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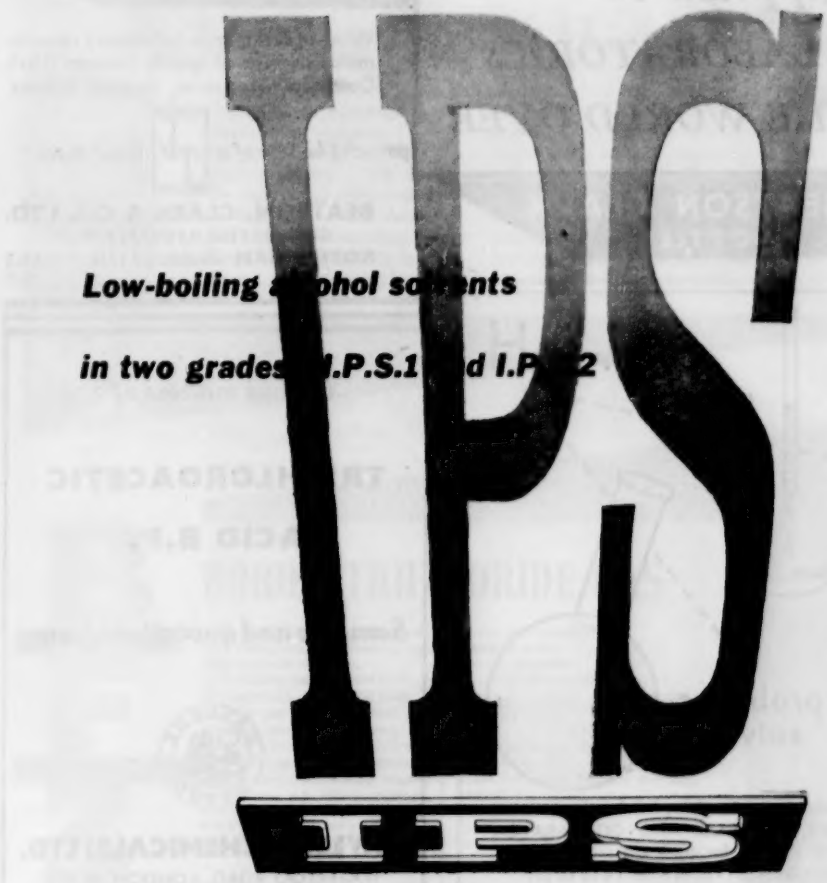


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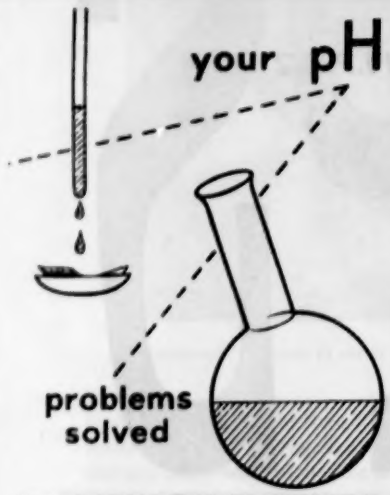


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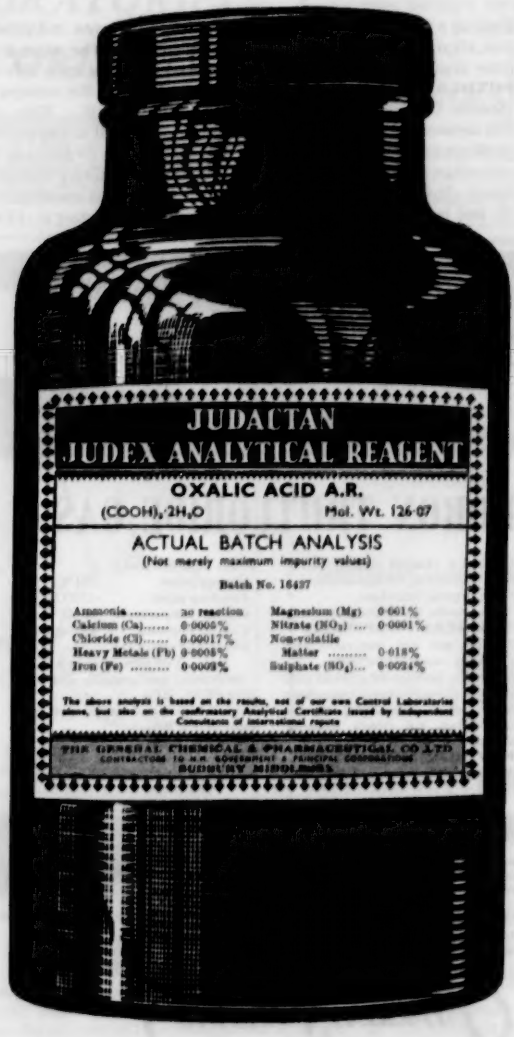


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## 229. *Aliphatic Hydroxylamines. Part II.* Autoxidation.*

By David H. Johnson, M. A. Thorold Rogers, and G. Trappe.

The autoxidation of aliphatic hydroxylamines in aqueous alkaline solution has been studied and the effect of catalysts has been demonstrated. Usually, 1½ or 2 atoms of oxygen are absorbed, and peroxidic material is formed. Part of this is hydrogen peroxide, but a part is organic, and progress towards the isolation of the pure organic component has been made. A mechanism, similar in some respects to that now generally accepted for olefinic autoxidation, is considered.

The ready autoxidation of aliphatic hydroxylamines, both primary, $R{\cdot}CH_2{\cdot}NH{\cdot}OH$, and secondary, $R{\cdot}CH_2{\cdot}N(OH){\cdot}CH_2R'$, has been known for a very long time. Indeed, it is difficult to avoid the formation of aldehyde, which has long been recognised as one of the products, during any manipulation of hydroxylamines, especially under alkaline conditions. Behrend and König [1] attribute the first observation to Beckmann. These authors, and Bamberger and Szolayski [2] examined the course of the reaction, and obtained a number of products; they also showed that hydrogen peroxide is formed. Since then the mechanism of autoxidation of the olefins has been extensively studied, but no re-investigation of the autoxidation of hydroxylamines has been undertaken in the light of the work of Farmer, Bolland, Gee, and others.

In this paper we report preliminary results which lead us to formulate a tentative mechanism for the autoxidation which resembles that now generally accepted for olefinic autoxidation. In the later stages our efforts have been concentrated on attempts to isolate the organic peroxides (which are posulated to be intermediates) in a pure state. Although we have failed to achieve this objective, we present evidence that organic peroxides are formed and can be isolated, though the conditions for isolating pure material in quantity require defining more closely.

We have worked almost entirely with *NN*-dialkylhydroxylamines, but no obvious difference in behaviour has been apparent in the few experiments carried out on the monoalkylhydroxylamines, and we believe that the same principles apply. All our autoxidations have been carried out in aqueous solution.

Many of the early experiments [2] were carried out on the readily available mono- or di-benzylhydroxylamine, and the aqueous suspensions were air-blown for long periods. The choice of dibenzylhydroxylamine was unfortunate as it is very insoluble, hence the oxidation is heterogeneous. Bamberger suggested that the oxidation of amines under certain conditions can proceed through the hydroxylamine, and appreciated [3] that the sequence was :

$$\text{Amine} \xrightarrow[\text{Slow}]{(a)} \text{Hydroxylamine} \xrightarrow[\text{Rapid}]{(b)} \text{Primary product} \xrightarrow[\text{Less rapid than } (b)]{(c)} \text{Secondary products}$$

but by the nature of his experiments he allowed the primary products to be further oxidised by stage (*c*) to a complex variety of end-products. We have attempted to overcome this obstacle, and have found that the simple dialkylhydroxylamines are autoxidised in aqueous solution or suspension at pH >7 at room temperature or at 37° at a rate which can be measured very conveniently by following the uptake of oxygen.

Our early experiments were carried out in a Warburg apparatus at 37°.† It was found that :

(1) The rate of oxygen uptake is a function of pH, being very slow at pH 7, and very rapid in the presence of sodium hydroxide (Fig. 1).

(2) In most runs the absorption of oxygen was 1½ atoms per molecule of hydroxylamine.

* Part I, *J.*, 1955, 769.

† In these experiments we were assisted by Dr. J. Madinavietia, whom we cordially thank.

[1] Behrend and König, *Annalen*, 1891, **263**, 210.

[2] Bamberger and Szolayski, *Ber.*, 1900, **33**, 3193.

[3] Bamberger, *Ber.*, 1902, **35**, 4293; Bamberger and Scheutz, *Ber.*, 1901, **34**, 2262; Bamberger and Seligman, *Ber.*, 1902, **35**, 4299; 1903, **36**, 685.

Fig. 1 is typical of many runs. In the presence of excess of sodium hydroxide the oxygen absorption–time curve shows a sharp inflection at a point corresponding to the absorption of 1½ atoms of oxygen, after which a slow absorption continues indefinitely. At pH 9 the curve is normally exponential to the 1½-atom absorption line, but (Fig. 1, curve *b'*) an occasional anomalous curve shows a different stoicheiometry, oxygen uptake ceasing at 1 atom per molecule of hydroxylamine.

(3) The normal curves found when operating at pH 7 or 9 follow first-order kinetics with

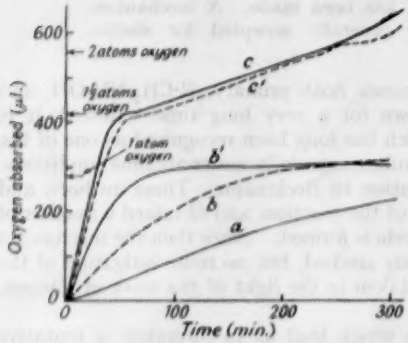



FIG. 1. *Autoxidation of dipropylhydroxylamine in the Warburg apparatus at 37°.*

(*a*) at pH 7, (*b*) at pH 9, (*b'*) anomalous curve at pH 9 as occasionally found, (*c*) with excess of sodium hydroxide, (*c'*) with excess of sodium hydroxide and added oxalic acid.

respect to the hydroxylamine as measured by oxygen uptake when the stoicheiometry is taken to require absorption of 1½ atoms of oxygen (Fig. 2).

(4) Dipropylhydroxylamine appeared to be autoxidised more rapidly than diethyl- or dibutyl-hydroxylamine, but this result may be without real meaning and a fortuitous consequence, not appreciated at that time, of traces of catalysts. The occasional anomalous or atypical result, such as that shown in Fig. 1, curve *b'*, can also be attributed to chance contamination by catalysts.

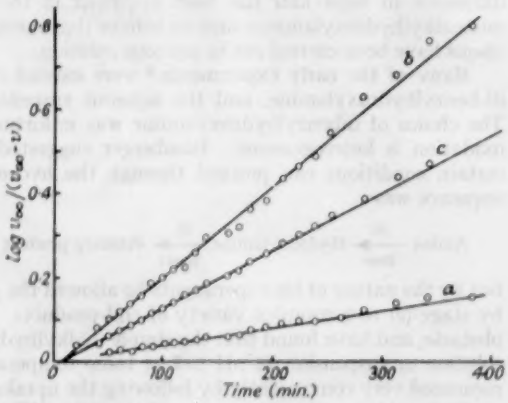



FIG. 2. *Logarithmic plot of rates of autoxidation at* pH 7 *of* (a) *diethyl-*, (b) *dipropyl-*, *and* (c) *dibutyl-hydroxylamine on the assumption of a stoicheiometry requiring the absorption of* 1½ *atoms of oxygen per molecule* (*experiments in Warburg apparatus at* 37°).

(5) There was no induction period in any experiment.

The scale of the early experiments in the Warburg apparatus precluded examination of the products. In later experiments we have carried out the autoxidation using a "Microid wrist-action" shaker, operated at room temperature, the oxygen absorption being measured in a burette. It was found that:

(6) Under these conditions the normal stoicheiometry was different from that found in the Warburg experiments at 37°, and 2 atoms of oxygen were now usually absorbed (Fig. 3), still without induction period.

(7) With this altered stoicheiometry the kinetics remain of first order with respect to the hydroxylamine (Fig. 4).

(8) The rate of oxygen uptake was enormously influenced by added trace-metal catalysts, notably copper and manganese.

(9) Oxygen uptake was entirely arrested by the addition of suitable metal-sequestering agents, but could be started again by the addition of further metal catalysts in excess of the sequestering agent.

(10) The autoxidised solution was peroxidic.

Some qualification and expansion is required for the statements (6)—(10). Just as in the Warburg experiments, results were not always reproducible. The normal stoicheiometry at room temperature would appear to involve 2 oxygen atoms, and indeed when the Warburg experiments were run at 22°, 2 atoms were absorbed (large-scale experiments at 37° were inconclusive). But every now and then, especially when catalysts were added, the stoicheiometry appeared to change to absorption of $1\frac{1}{2}$ atoms or to some intermediate figure. The rate of autoxidation also changed from time to time, without affecting the stoicheiometry. Slow absorption was found in one instance to be a result of

FIG. 3. *Autoxidation of dipropylhydroxylamine hydrogen oxalate (0·4271 g.) at 23° and 755·7 mm. in laboratory shaker in presence of excess of sodium hydroxide.*

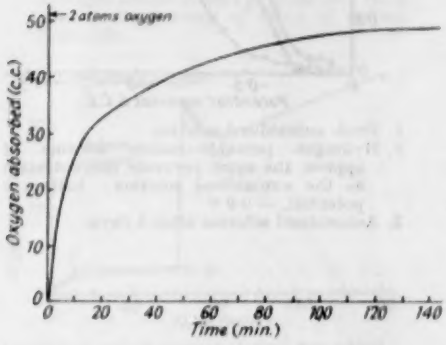
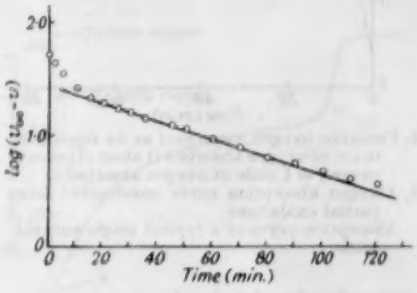


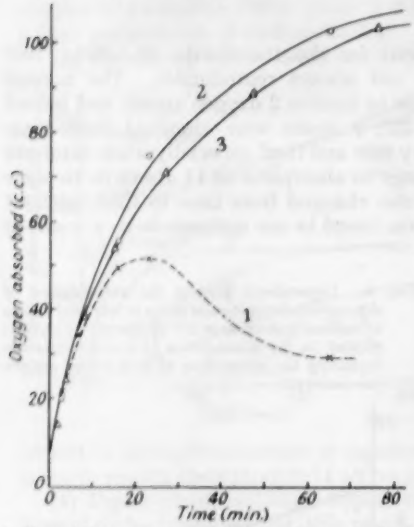

FIG. 4. *Logarithmic plot of the autoxidation of dipropylhydroxylamine in the presence of excess of sodium hydroxide at 23° in laboratory shaker, plotted on the assumption of a stoicheiometry requiring the absorption of 2 atoms of oxygen per molecule.*




using insufficiently pure oxygen, but other suspected influences were shown to be unimportant; thus, light had no obvious effect on the rate, nor did addition of glass wool or replacement of the glass by a Polythene container. Some further experiments with catalysts are discussed below.

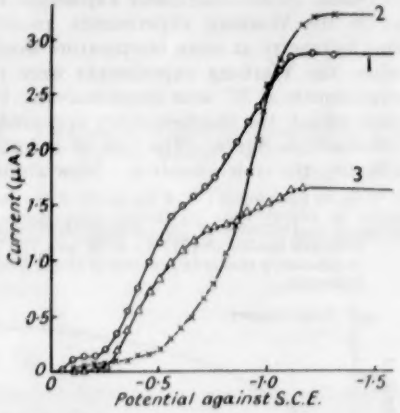
The production of peroxidic material under conditions of controlled oxygen uptake has obvious bearing on the mechanism. We have therefore examined the peroxidic material with care, and conclude that the peroxide activity is due to a mixture of hydrogen peroxide and organic peroxide. When peroxide content is plotted against oxygen absorption (Fig. 5) it is seen that the curve bears a striking resemblance to that found in the autoxidation of benzaldehyde (see, *e.g.*, Waters, "The Chemistry of Free Radicals," Oxford, 1946, p. 237). The peroxide reaches a maximum at a point on the curve between 10 and 30% of the final oxygen absorption. The peroxide content of autoxidised solutions slowly falls when kept, but not to zero, particularly with fully autoxidised solutions. The possibility that the residual activity was organic peroxide, and the labile material was hydrogen peroxide, was supported by polarographic analysis (Fig. 6), the fully autoxidised solution giving a wave which is clearly attributable in part to hydrogen peroxide (half-wave potential −0·9 v; see Kolthoff and Lingane, "Inorganic Polarography," Interscience, New York, 2nd edn., 1952, p. 447), but which had a shoulder at much lower potential. As the total peroxide (iodine titration) decayed, so the shoulder became relatively more and more important, and the wave attributed to hydrogen peroxide less and less dominant. The half-wave potential of the residual peroxide was estimated to lie between −0·40 and −0·45 v (see Fig. 6, curve 3).

FIG. 5. *Comparison of the peroxide content with oxygen absorption during autoxidation of dipropylhydroxylamine at 23° in the presence of excess of sodium hydroxide.*




1, Peroxidic oxygen, expressed as its equivalent in c.c. of oxygen absorbed (1 atom of peroxy-oxygen ≡ 1 mole of oxygen absorbed).
2, Oxygen absorption curve constructed from partial oxidations.
Absorption curve of a typical single autoxidation.

FIG. 6. *Polarogram of autoxidised solution of dipropylhydroxylamine at 23° in presence of sodium hydroxide.*




1, Fresh autoxidised solution.
2, Hydrogen peroxide-oxalate solution of approx. the same peroxide concentration as the autoxidised solution; half-wave potential, — 0·9 v.
3, Autoxidised solution after 3 days.

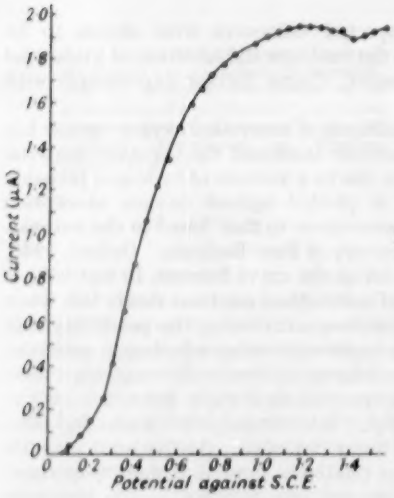


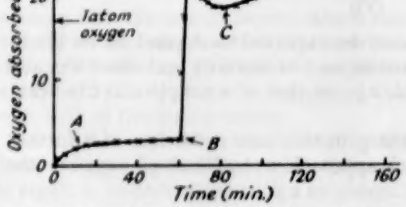

FIG. 7. *Polarogram of the peroxide from dipropylhydroxylamine, after distillation; half-wave potential,* — 0·425 v.

It is surprising that the shoulder only appears in nearly or quite fully autoxidised solutions. It would be unwise to deduce from this that no organic peroxide is present under conditions of partial oxidation, but we confined our further attempts to isolate the peroxide to fully autoxidised material (see Experimental section). They were only partly successful; we isolated a volatile material, boiling up to 70°, highly peroxidic, with a half-wave potential of −0·42 v (Fig. 7), and no sign of the characteristic hydrogen peroxide wave, and giving propionaldehyde and monomethylhydroxylamine on acidification, but analyses were unsatisfactory; organic peroxide is undoubtedly present, but its isolation pure must await detailed physicochemical study, which is being undertaken elsewhere.

*Catalysts in the Autoxidation Process.*—The action of copper and manganese in promoting autoxidation of nitrogenous substances has analogies; thus Audrieth and Mohr [4] have shown the great effect of copper in promoting autoxidation of hydrazine.

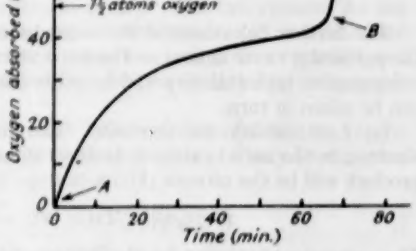
Cobalt, which has little or no positive catalytic effect, causes a characteristic trough in the absorption curve at or about the point of maximum absorption (Fig. 8). This is found regardless of the order in which the copper and cobalt are added, or of the stage in the oxidation. The failure of cobalt to catalyse aqueous autoxidations is not unexpected.[5] Audrieth and Mohr [4] found vanadate to be a good catalyst for hydrazine oxidation; we find it to be without effect in our autoxidations. Sulphite had no effect, but β-naphthol had a marked positive effect, and was itself partially oxidised to yellow-brown material of unknown structure. Baryta would appear at first sight to be acting merely as an alkali, but with monopropylhydroxylamine at room temperature and with dipropylhydroxylamine at 37° it showed a remarkable break in the rate of oxygen uptake, the absorption suddenly increasing very considerably, at which point the solution became yellow (Fig. 9).

FIG. 8. *Autoxidation of dipropylhydroxylamine hydrogen oxalate (0·2812 g.) at 20° and 761·5 mm. in the presence of excess of sodium hydroxide.*




At point *A*, cobalt acetate (1 mg.) was added.
At point *B*, copper acetate (1 mg.) was added.
(The gas evolution leading to trough *C* is characteristic of the presence of cobalt.)

FIG. 9. *Autoxidation of monopropylhydroxylamine at 22·5° and 774 mm. in the presence of excess of baryta.* (The curve for the autoxidation of dipropylhydroxylamine at 37° in the presence of excess of baryta is similar.)




At point *A*, barium peroxide began to be precipitated.
At point *B*, the solution darkened considerably.

*Mechanism.*—In earlier work it has been assumed that two hydrogen atoms are removed by a mechanism unspecified to give (I), which is presumed to hydrolyse to the aldehyde and

$$R{\cdot}CH_2{\cdot}N(O){:}CHR \quad (I) \qquad R{\cdot}CH_2{\cdot}N(OH){\cdot}CH(OH)R \quad (II) \qquad R{\cdot}CH_2{\cdot}N(OH){\cdot}CH(O{\cdot}OH)R \quad (III)$$

to hydroxylamine. This dehydrogenation process is of the type postulated by Wieland [6] for many biological oxidations, including the oxidation of amines and amino-acids.

[4] Audrieth and Mohr, *Ind. Eng. Chem.*, 1951, **43**, 1774.
[5] Bawn and Williamson, *Trans. Faraday Soc.*, 1951, **47**, 743.
[6] Wieland, "On the Mechanism of Oxidation," Yale Univ. Press, 1932, p. 87; see also Green, "Mechanism of Biological Oxidation," Cambridge, 1940, pp. 87, 143.

The mechanism of the autoxidation of hydroxylamines can be considered in two stages:

(1) *The primary process.* Our results show: (*a*) That the rate of autoxidation is a function of pH, so that it is reasonable to assume that an ionic species is involved. (*b*) That the rate of autoxidation is trace-metal catalysed, and because of this, and by analogy with other autoxidation systems, we can say that a radical or one-electron system is involved. These two criteria can be accommodated satisfactorily in the equations

$$R{\cdot}CH_2{\cdot}N(OH){\cdot}CH_2R \xrightarrow{OH^-} R{\cdot}CH_2{\cdot}N(O^-){\cdot}CH_2R \text{ (IV)} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

$$R{\cdot}CH_2{\cdot}N(O^-){\cdot}CH_2R \xrightarrow[+H^+]{O_2} R{\cdot}CH_2{\cdot}N(O\cdot){\cdot}CH_2R \text{ (V)} + HOO\cdot \quad . \quad . \quad . \quad . \quad (2)$$

There is ample evidence for the formation of radicals such as (V), and, provided that there is no α-hydrogen atom, they are stable. For instance, Teuber and Jellinek [7] have reviewed the literature on Frémy salt $(^-O_3S)_2NO\cdot$; Gilman and McCrachen [8] examined the radical $Ph_2NO\cdot$, and Banfield and Kenyon [9] made the compound

$$\begin{matrix} CMe_2{\cdot}CH_2{\cdot}CMe \\ Ph{\cdot}N{\cdot}O\cdot \quad Ph{\cdot}N{\rightarrow}O \end{matrix}$$

(2) *Subsequent reaction.* When there is a free α-hydrogen atom a nitrosyl compound is not isolated, and it follows from the products of the oxidation that an α-carbon atom must be oxidised. One of the simplest of several processes which will accommodate these facts is:

$$\underset{(V)}{R{\cdot}CH_2{\cdot}N(O\cdot){\cdot}CH_2R} + \underset{(IV)}{R{\cdot}CH_2{\cdot}N(O^-){\cdot}CH_2R} \longrightarrow \underset{(VI)}{R{\cdot}CH_2{\cdot}N(O^-){\cdot}\dot{C}HR} + R{\cdot}CH_2{\cdot}N(OH){\cdot}CH_2R \quad (3)$$

The further behaviour of the radical-ion (VI) may be expected to depend on its life in the particular environment. The two extreme possibilities, low stability and short life, and comparative high stability and long life (compared, *e.g.*, to that of a simple alkyl radical), can be taken in turn.

(a) *Low stability and short life.* The probable step in this case is the loss of a further electron to the metal catalyst, to any radicals which approach, or to dissolved oxygen; the product will be the nitrone (I):

$$R{\cdot}CH_2{\cdot}N(O^-){\cdot}\dot{C}HR \text{ (VI)} \xrightarrow{-e} R{\cdot}CH_2{\cdot}N(\rightarrow O){=}CHR \text{ (I)} \quad . \quad . \quad . \quad . \quad . \quad (4)$$

This, presumably, must be the mechanism when hydroxylamines are oxidised by metal oxides in organic media [if, indeed, (VI) is an intermediate in such cases]. The resultant overall process is the familiar dehydrogenation oxidation, and no organic peroxide should be formed.

(b) *High stability and long life.* If the system is reluctant to lose its unpaired electron it may survive long enough to combine with other radicals, *viz.*, hydroperoxy, oxygen, and possibly hydroxyl; dimerisation is improbable because of the negative charge.

(i) Combination with HO·O· will form the hydroperoxide (III) in its ionised form (III*a*) in one step:

$$R{\cdot}CH_2{\cdot}N(O^-){\cdot}\dot{C}HR \text{ (VI)} + HO{\cdot}O\cdot \longrightarrow R{\cdot}CH_2{\cdot}N(O^-){\cdot}CH(O{\cdot}OH)R \text{ (III}a\text{)} \quad . \quad . \quad (5)$$

(ii) Combination with oxygen will give the radical (VII), and from this point it seems safe to draw on the analogy with the currently accepted mechanism for olefin autoxidation:

$$R{\cdot}CH_2{\cdot}N(O^-){\cdot}\dot{C}HR \text{ (VI)} + O_2 \longrightarrow R{\cdot}CH_2{\cdot}N(O^-){\cdot}CH(OO\cdot)R \text{ (VII)} \quad . \quad . \quad (6)$$

$$R{\cdot}CH_2{\cdot}N(O^-){\cdot}CH(O{\cdot}O\cdot)R + R{\cdot}CH_2{\cdot}N(O^-){\cdot}CH_2R \longrightarrow R{\cdot}CH_2{\cdot}N(O^-){\cdot}CH(O{\cdot}OH)R \text{ (III}a\text{)} + R{\cdot}CH_2{\cdot}N(O^-){\cdot}\dot{C}HR \text{ (VI)} \quad . \quad (7)$$

(iii) Combination with HO·, if present, would lead to the formation of the hydroxy-hydroxylamine (II), the hydrated form of the nitrone. The existence of significant amounts

[7] Teuber and Jellinek, *Chem. Ber.*, **1952**, **85**, 95.
[8] Gilman and McCrachan, *J. Amer. Chem. Soc.*, 1927, **49**, 1052.
[9] Banfield and Kenyon, *J.*, 1926, 1612.

of hydroxyl radical would presumably depend on the presence of metal catalysts, able to react suitably with hydrogen peroxide; thus $Fe^{++}$ would give a reagent of Fenton type:

$$R{\cdot}CH_2{\cdot}N(O^-){\cdot}\dot{C}HR + HO{\cdot} \longrightarrow R{\cdot}CH_2{\cdot}N(O^-){\cdot}CH(OH)R \longrightarrow R{\cdot}CH_2{\cdot}N(O){=}CHR \quad (8)$$

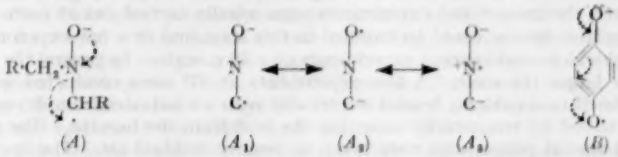
A further possible route to a hydroperoxide is:

$$R{\cdot}CHO + R{\cdot}CH_2{\cdot}NH{\cdot}OH \overset{OH^-}{\rightleftharpoons} R{\cdot}CH_2{\cdot}N(O^-){\cdot}CH(OH)R + H_2O_2 \longrightarrow R{\cdot}CH_2{\cdot}N(O^-){\cdot}CH(O{\cdot}OH)R\ (IIIa) + H_2O \quad (9)$$

but we have found only traces of organic peroxide when hydrogen peroxide reacts with the aqueous alkaline condensation product of propionaldehyde and monoalkylhydroxylamine.

Fortunately, Rogers [10] has found independent evidence of (VI) as an intermediate so that this last possibility does not represent the main reaction sequence and the evidence is strongly in favour of the concept of (VI) being relatively stable.

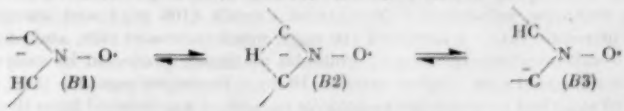
*The Structure and Stability of the Radical-ion* (VI).—Having postulated a stability for the radical-ion (VI) which is greater than that of typical alkyl radicals, some structural rationalisation of the stability is required. In terms of resonance this can be expressed as (*A*), this representing the canonical forms ($A_1$), ($A_2$), ($A_3$). Such a system has some



(*A*) ($A_1$) ($A_2$) ($A_3$) (*B*)

analogy with the semiquinones, which may be written as in (*B*). The two systems are not entirely analogous, however, because the radical-charge separation in (*B*) is even (four atoms), whereas in (*A*) it is odd (one atom); but the *p*-phenylene unit is here regarded as a whole. It is not, of course, suggested that the radical-ion (VI) has a stability comparable with that of the semiquinones.

A further structure which might contribute to the enhancement of stability can be derived from ($A_2$) and ($A_3$), when the negative charge on the carbon atom may be expected to result in co-ordination with a neighbouring hydrogen atom:



(*B*1) (*B*2) (*B*3)

There is some experimental support for these tautomeric forms. It has been found that *N*-ethyl-*N*-methylhydroxylamine and *N*-methyl-*N*-propylhydroxylamine, autoxidised under the conditions described, give substantially only formaldehyde. Now this could be the result if the radical oxidation stage (equation 3) were much more rapid on a methyl than on an ethyl or propyl radical. But if this were the case the rate of autoxidation of dimethylhydroxylamine should be much greater than that of the higher homologues. For reasons already given, no accurate figures for the rates can yet be given, but certainly the rate of autoxidation of dimethylhydroxylamine is not greatly different from that of the higher homologues, and the difference is not enough to account for the great preponderance of formaldehyde found in the oxidation of the unsymmetrical hydroxylamines. But if (*B*2) represents a further canonical form of (VI), the aldehyde formed from the oxidation of an unsymmetrical hydroxylamine should not be consequent on relative oxidation rates of the two alkyl groups.

## Experimental

*Materials.*—The dialkylhydroxylamines were made by the methods of Part I (*loc. cit.*). They were normally stored and used as the hydrogen oxalates, these being the simplest non-hygroscopic salts. Experiment showed that oxalic acid has no effect on the oxidation rates

[10] Rogers, *Chem. and Ind.*, 1953, 1033; and forthcoming publication.

(*e.g.*, Fig. 1, effect of adding further oxalic acid). Monopropylhydroxylamine was made by Beckmann's method.[11] Rates of autoxidation varied with the batch, probably owing to presence of traces of metal catalysts. Sodium hydroxide solutions were generally made by dilution of filtered concentrated solutions of flake sodium hydroxide; solutions made from commercial pellets usually gave slow autoxidation rates. Oxygen was of pure anæsthetic quality.

*Autoxidation in the Warburg Apparatus.*—Unless otherwise stated all experiments were carried out at 37°. Agitation was kept at a rate which experiment showed was not critical, and small alterations had no effect on the oxygen-absorption curve. All flasks contained 2 c.c. of solution of the oxalate, and the centre cup was filled with 2N-sodium hydroxide. Sodium hydroxide or buffer solution (0·2 c.c.) was added from the side arm. For experiments at pH 7, phosphate buffer (0·25M) was made up in 0·25N-sodium hydroxide, and for experiments at pH 9, borax buffer (0·3M) in 0·25N-sodium hydroxide was used. The available alkali neutralised the oxalic acid, and trial showed that the appropriate pH was attained and maintained throughout the experiment.

Normal practice [12] for equilibration and calculation was observed. The gas phase was air, and calculation showed that the oxygen depletion was negligible.

*Autoxidation in the Larger-scale Apparatus.*—A 250 c.c. flask connected to a gas burette was shaken vigorously by a "Microid wrist-action" shaker; failure to shake vigorously enough accentuates the initial deviation from the straight-line first-order plot (Fig. 4). The apparatus was not kept in a thermostat and experiments were usually carried out at room temperature; up to 1 g. of hydroxylamine could be handled in this way, and in a few experiments, a larger shaker was used which would permit experiments on a 10 g. scale. In general the concentration was higher the larger the scale. A few experiments at 37° were conducted with the whole apparatus enclosed in a cabinet, heated electrically with an industrial fan dryer. The alkali, or buffer, was added by temporarily removing the lead from the burette. The gas phase was oxygen but no special precautions were taken to remove residual air. The apparatus was in diffuse daylight.

*Effect of Catalysts.*—Aqueous solutions of copper acetate have shown marked acceleration of oxygen uptake at all pH studied; in concentrations of about 1 : 100,000 the oxygen absorption in the presence of sodium hydroxide was too rapid for measurement, and the almost imperceptible uncatalysed absorption at pH 7 was much increased. The stoicheiometry and general shape of the absorption curve with copper and sodium hydroxide present were not regularly reproducible.

In a series of autoxidations, the rate of absorption was noted when 2N-sodium hydroxide (3 c.c.) was added to dipropylhydroxylamine (400 mg. of hydrogen oxalate) in water (30 c.c.) containing the following substances: Manganese dioxide (100 mg.) and manganese acetate (1 mg.), much increased rate; β-naphthol (10 mg.), much increased rate, and oxidation of the β-naphthol to a yellow-brown substance; colloidal platinum, protected by polyvinyl chloride (100 mg.), much increased rate. Silver nitrate (10 mg.), hydrogen peroxide (30%; 1 mg.), and benzoic acid (100 mg.) had no effect (no hydroxybenzoic acid was isolated from the last of these). Benzoyl peroxide (10 mg.), sodium sulphite (10 mg.), ferrous sulphate (10 mg.), and ammonium vanadate (1 mg.) appeared to depress the rate somewhat. Cobalt acetate (1 mg.) appeared to depress the rate (Fig. 8) and, in certain circumstances already discussed, caused a break in the curve.

When the autoxidation of dipropylhydroxylamine (280 mg.) in aqueous solution (30 c.c.) at pH 9, containing 1 mg. of copper acetate, was interrupted by addition of the following metal-sequestering agents, the oxygen uptake ceased: salicylaldoxime (15 mg. in aqueous alcohol), sodium diethyldithiocarbamate (30 mg. in water), and "Cupron" (30 mg. in ethanol), in the first two cases, abruptly, in the last, slowly. The uncatalysed autoxidation was also inhibited by these reagents. In each case the addition of copper ions in large molar excess over the sequestering agent resulted in further oxygen uptake.

The experiment represented by Fig. 2 demonstrates the first-order character of the reaction but the linearity is not much affected by the stoicheiometry. The experiment was made before the importance of the catalytic effect was fully appreciated, and the relative rates must be taken with reserve. Nevertheless, because dipropylhydroxylamine appeared to be most rapidly oxidised, it has been selected for the later experiments.

[11] Beckmann, *Annalen*, 1909, **365**, 205.

[12] Dixon, "Manometric Methods as applied to the Measurement of Cell Respiration and Other Processes," Camb. Univ. Press, 1943.

*Hydrogenation of the Product of Autoxidation of Dipropylhydroxylamine.*—Dipropylhydroxylamine hydrogen oxalate (501·9 mg.) was autoxidised in aqueous solution (30 c.c.) containing 2N-sodium hydroxide (4·0 c.c.). When uptake of oxygen was complete (2 atoms per molecule) the solution was hydrogenated with 5% palladium on carbon catalyst; 105·1 c.c. of hydrogen were taken up (Calc. for 4H per mole: 120·4 c.c.). The filtered solution was steam-distilled, and from the distillate was obtained dipropylamine as its picrate (15 mg.), m. p. 97—98°.

*Hydrogenation of Monopropylhydroxylamine–Propionaldehyde Mixture.*—A solution of the hydroxylamine hydrogen oxalate (402 mg.) in water (30 c.c.) containing 2N-sodium hydroxide (3·0 c.c.) was added dropwise to a solution of propionaldehyde (0·8 g.; freshly distilled) in water (20 c.c.), with stirring. At the end of 1 hr. the solution was not appreciably reducing in the triphenyltetrazolium chloride test. The solution was hydrogenated in the same way as in the previous experiment, and by steam-distillation there was isolated dipropylamine as its picrate (15 mg.), m. p. 97—98°.

Aqueous alkaline solutions of monopropylhydroxylamines are autoxidised as readily as those of the dialkyl series. When an alkaline solution of monopropylhydroxylamine was mixed with propionaldehyde, and the mixture kept for 1 hr., it was autoxidised very slowly. This is consistent with the suggestion that there has been reaction to form $Pr{\cdot}N[CH(OH){\cdot}CH_2{\cdot}CH_3]{\cdot}OH$. When copper (as acetate) was added, autoxidation proceeded more rapidly.

*Determination of Total Peroxide in Autoxidised Solutions of Dipropylhydroxylamine.*—A stream of carbon dioxide was passed through glacial acetic acid ("AnalaR"; 25 c.c.) in a 250 c.c. conical flask for 5 min. With the gas stream continuing, sodium iodide solution (60%; 2 c.c.) was added, followed by the peroxide solution (10 c.c.) under examination. The flask was tightly stoppered and kept for 30 min., and the liberated iodine was titrated with 0·1N-sodium thiosulphate. Blank titres were always less than 0·1 c.c. All determinations were carried out in duplicate, and agreement was satisfactory.

*Polarographic Measurements.*—A Tinsley automatic polarograph was used, with saturated calomel reference electrode. The peroxidic solution (0·5 c.c.), adjusted previously to pH 8 by saturation with carbon dioxide (conveniently by the addition of solid carbon dioxide), was added to 0·1N-lithium chloride solution (15 c.c.) in the cell, and dissolved oxygen was removed by passing a slow stream of nitrogen for 20 min. immediately before a determination. Condenser current blanks were obtained with appropriate blank solutions.

*Hydrogen Peroxide and Organic Peroxide Content of Fully Autoxidised Dipropylhydroxylamine.*—Three typical experiments illustrate the results, which were qualitatively reproducible:

*Solution A*: Dipropylhydroxylamine hydrogen oxalate solution (0·24 M; 20 c.c.) was mixed with 0·5N-sodium hydroxide (40 c.c.) and autoxidised to completion; 1·86 g.-atoms of oxygen were taken up, $t_{\frac{1}{2}} = 28$ min. Total peroxide was determined at once, and again after 3 and 6 days. The corresponding polarograms are shown in Fig. 6, which also shows the polarogram for a solution of hydrogen peroxide of similar concentration (trial and error).

*Solution B*: Dipropylhydroxylamine hydrogen oxalate solution (0·72 M; 20 c.c.) was mixed with N-sodium hydroxide (60 c.c.) and autoxidised to completion; 1·91 g.-atoms of oxygen were taken up. The low value for peroxide content (see Table 1) is characteristic of the more concentrated solution.

TABLE 1. *Peroxidic oxygen content of typical autoxidised solutions of dipropylhydroxylamine.*

| | Atoms peroxy-O / Mols. hydroxylamine | | Atoms peroxy-O / Mols. hydroxylamine |
|---|---|---|---|
| Solution A: freshly autoxidised | 0·290 | Solution B: freshly autoxidised | 0·06 |
| after 3 days | 0·189 | Solution C: freshly autoxidised | 0·21 |
| after 6 days | 0·165 | | |

*Solution C*: A typical chance slow oxidation. (The quality of the potassium hydroxide used was considered to be responsible.) Dipropylhydroxylamine hydrogen oxalate solution (0·097M; (50 c.c.) mixed with 2N-potassium hydroxide (10 c.c.) absorbed 1·83 g.-atoms of oxygen per mole of hydroxylamine, $t_{\frac{1}{2}} = 54$ min. The peroxidic content was rather lower than in the typical fast oxidation.

*The Peroxide Content and Characteristic Polarograms of Partially Autoxidised Dipropylhydroxylamine.*—It was considered impracticable to determine oxygen uptake and peroxidic content at intervals during a single experiment, so that a number of autoxidations, in identical conditions, were used to make a composite curve. Values of $t_{\frac{1}{2}}$ were very similar in all

experiments, in which the same apparatus and stock solutions were used throughout the series. In each case dipropylhydroxylamine oxalate (1·0 g.) in water (20 c.c.) and N/2-sodium hydroxide (40 c.c.) was autoxidised to the degree required, whereupon the shaking was stopped and the solution adjusted to pH 8 by solid carbon dioxide and made up to 100 c.c. with water. In order to obtain a closer approximation of the peroxide content at the time of interrupting the autoxidation, the determination was repeated on each sample several times, at intervals, whereby an allowance for the decay of the peroxide was made by back extrapolation. In Fig. 5 a composite and a typical oxygen-uptake curve are shown, together with a composite peroxide-content curve corrected for decay. Polarograms taken at each point showed only the characteristic wave for hydrogen peroxide until almost complete oxidation (1·73 atoms of oxygen per molecule), when the shoulder associated with organic peroxide appeared (Fig. 6).

*Separation of Organic Peroxide and Hydrogen Peroxide.*—It was shown that, on distillation under reduced pressure, and unlike hydrogen peroxide under the same conditions, the peroxidic activity of a fully autoxidised dipropylhydroxylamine solution at pH 8 (sodium hydrogen carbonate) passed over partially into the distillate, which now gave a polarogram entirely lacking the wave due to hydrogen peroxide, but showing a new wave with a half-wave potential of −0·425 V (Fig. 7). Dipropylhydroxylamine hydrogen oxalate (10·0 g.) in water (200 c.c.) and 0·5N-sodium hydroxide (400 c.c.), autoxidised to completion, was saturated with carbon dioxide by solid carbon dioxide and partially distilled (155 c.c. collected) at 11 mm., a trap cooled to about −40° being used. The degree of concentration obtained is shown by the titre (c.c. of 0·1N-sodium thiosulphate) per c.c. of the original solution (3·20), the distillate (8·54), and the residue (1·38).

*Isolation of Organic Peroxide.*—The isolation from the distillate was normally carried out at once, but the peroxidic activity and the characteristic polarographic wave did not alter during several days at laboratory temperature. The distillate from two oxidations, each on 10 g., was extracted repeatedly with ether (20 × 20 c.c.) and the combined extracts were dried ($K_2CO_3$); hydrogen peroxide in ether is completely removed by this treatment. The ether was removed by passing a stream of dry air through until the issuing gases showed marked peroxidic activity to acidified starch–iodide paper. The gas stream was passed into a receiver, cooled by a freezing mixture, and as it was markedly peroxidic the process was repeated on the condensate. The combined residues were fractionated at atmospheric pressure through a short column (Table 2).

TABLE 2. *Typical fractionation of organic peroxide from dipropylhydroxylamine.*

| Fraction | B. p. | Remarks | Fraction | B. p. | Remarks |
|---|---|---|---|---|---|
| 1 | Below 38° | Ether | 4 | 48·5—67° | Strongly peroxidic |
| 2 | 39—41·5 | Mainly ether | 5 | 67—71 | 0·16 g., strongly peroxidic |
| 3 | 41·5—48·5 | ,, | 6 | 71—72 | 0·23 g., ,, ,, |

The material from fractions 5 and 6, and similar material from several other preparations of a similar nature in some of which the concentration procedure was omitted, was extraordinarily volatile. It had a sharp pungent smell, and when it was warmed a carbylamine-like smell developed; propionaldehyde-like smell was not detected until the liquid was acidified, but it then developed immediately. It showed little reducing action towards triphenyltetrazolium chloride but, when acidified, immediately developed strong reducing characteristics. It gave a strong purple-red ferric reaction in methanol, and a polarogram identical with that of the undistilled material (half-wave potential = −0·425 V). On reaction with 2 : 4-dinitrophenylhydrazine in warm 2N-hydrochloric acid, the dinitrophenylhydrazone of propionaldehyde was immediately precipitated (m. p. and mixed m. p.), and with aqueous oxalic acid the oxalate of monopropylhydroxylamine (m. p. and mixed m. p.) was isolated. When a mixture of acrylonitrile and the peroxide was warmed on the steam-bath, the acrylonitrile polymerised.

Many attempts were made to get reproducible analytical results which could be correlated with any imagined peroxide structure. To avoid loss during the sweeping-out operation in the nitrogen determination, the sample was either cooled in carbon dioxide in a U-tube during this stage, or was sealed in a capillary, which was subsequently crushed by means of a plunger.* Despite these precautions, all results for nitrogen, except one, have been under half the expected, and results generally were very erratic. Azeotropes with ether are a possible complication.

Other methods of isolation were tried. The preliminary separation from hydrogen peroxide

* The modifications to the standard procedure were kindly carried out by Mr. R. Rothwell, whom we thank.

may be omitted, and the total oxidation product neutralised and extracted with ether, and the hydrogen peroxide extracted by anhydrous potassium carbonate. The method has no advantage. Finally, attempts were made to isolate the organic peroxide as sodium salt. The autoxidised solution was evaporated to dryness at about 0·1 mm., leaving a peroxidic residue. Alternatively, the water-distilled material (see p. 1102) was treated with excess of sodium hydroxide and evaporated under reduced pressure. In both cases the residue was strongly peroxidic, and remained so for months, but it did not show any sign of the polarographic wave associated with organic peroxide. It contained nitrogen, and had some of the properties to be expected, but attempts to purify it failed.

*Autoxidation of Ethylmethyl- and of Methylpropyl-hydroxylamines.*—The autoxidation was carried out, without added catalyst, at room temperature on the 0·5 g. scale. The resultant solution was made slightly acid, and mixed with an aqueous alcoholic solution of dimedone. The mixture was kept overnight, and the precipitate was then collected and dried. It was treated with glacial acetic acid under the conditions for separating formaldehyde from its homologues.[13] In each case only a trace of the higher homologue was isolated, and the anhydride of the formaldehyde derivative was obtained in a pure state.

The authors thank their colleagues and Professor C. E. H. Bawn for many helpful discussions.

IMPERIAL CHEMICAL INDUSTRIES LIMITED,
DYESTUFFS DIVISION, RESEARCH DEPARTMENT,
HEXAGON HOUSE, BLACKLEY, MANCHESTER, 9. [*Received, March 26th*, 1955.]

[13] Hopkin and Williams Ltd., "Organic Reagents for Organic Analysis," 1944.

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## 230. 2-*Mercaptoglyoxalines. Part X.* The Acylation of 2-Mercaptoglyoxalines.*

By ALEXANDER LAWSON and H. V. MORLEY.

2-Mercaptoglyoxalines, which show an absorption maximum in the 2600 Å region due mainly to contributions from the thione form, are acylated in basic media at a nitrogen atom, probably as a result of rearrangement of the *S*-acyl isomer first formed. In the case of acyl derivatives such as 1-ethoxycarbonyl-2-mercapto-4- or -5-methylglyoxaline (III), possible reasons for the bathochromic shift in the absorption maximum from the 2600 to the 3050 Å region are advanced.

INTEREST in the acylation of 2-mercaptoglyoxalines arose owing to the necessity of protecting the sulphur and the nitrogen atom of 2-mercaptohistidine during the methylation which led to the synthesis of ergothioneine by Heath, Lawson, and Rimington.[1] The product obtained by the action of ethyl chloroformate on 2-mercaptohistidine was designated as 2-ethoxycarbonylthiohistidine since methylation affected only the side-chain amino-group and the ultraviolet absorption failed to show a maximum at *ca.* 2600 Å (in ethanol) believed to be characteristic of the mercapto-group in 2-mercaptoglyoxalines. The same workers also investigated the use of benzylation and acetylation as protecting groups.

The effect of acylation on the 2-mercaptoglyoxaline structure has been investigated by using 2-mercapto-4(5)-methylglyoxaline [2] (I). With ethyl chloroformate in pyridine this gave an ethoxycarbonyl derivative for which the structures (II) and (III) (or tautomers thereof) are possible. The ultraviolet spectrum did not show the characteristic peak at 2600 Å and thus, according to the interpretation of Heath *et al.*,[1] structure (II) seemed indicated.

The formation of 2-ethylthio-4(5)-methylglyoxaline when this substance was heated did not provide evidence of structure, for, although Jones [3] reports ethylation of the sulphur

* Part IX, *J.*, 1955, 1695.

[1] Heath, Lawson, and Rimington, *J.*, 1951, 2215.
[2] Bullerwell and Lawson, *J.*, 1951, 3030.
[3] Jones, *J. Amer. Chem. Soc.*, 1952, **74**, 1084.

atom on heating 4(5)-ethoxycarbonyl-2-mercaptoglyoxaline, Dixon [4] showed that *S*-ethoxycarbonylthiuronium chlorides also give *S*-alkyl derivatives when heated.

It is known that under certain conditions oxidation of 2-mercaptoglyoxalines leads to disulphides.[5] Oxidation of the compound (II or III) with nitric acid gave a crystalline disulphide, $C_{14}H_{18}O_4N_4S_2$ (IV), which could have been produced only from structure (III). The disulphide on hydrolysis lost the ethoxycarbonyl groups and gave an amorphous basic substance (V) (probably polymeric) having the composition of a disulphoxide.

The correctness of the structure 1-ethoxycarbonyl-2-mercapto-4- or -5-methylglyoxaline (III) was confirmed by methylation, followed by hydrolysis to remove the ethoxycarbonyl group. The basic product (VI) was identical with that obtained by the direct methylation of 2-mercapto-4(5)-methylglyoxaline (I) which according to Marckwald [6] would be expected to give an *S*-methyl derivative. The *S*-alkyl structure was confirmed by the preparation from the corresponding ethylthio-derivative of a bromo- and an iodo-derivative with no sign of disulphide formation.

From the above results it is clear that failure to show absorption in the 2600 Å region is not sufficient evidence for deciding between *N*- and *S*-acylation in 2-mercaptoglyoxalines.

It seems probable that 1-ethoxycarbonyl-2-mercapto-4- or -5-methylglyoxaline (III) arises by migration of the acyl group from the sulphur to the nitrogen atom. Such a rearrangement would account for the same substance's being obtained from the mercaptoglyoxaline and ethyl chloroformate in sodium hydroxide solution, *i.e.*, under conditions which would favour *S*-substitution. Moreover, when the ethoxycarbonylation is done at room temperature in a non-basic medium, a hydrochloride of a base giving a picrate is obtained. This base must be the *S*-ethoxycarbonyl isomer (II) since the known *N*-ethoxycarbonyl derivative (III) is non-basic and does not give a picrate. The above hydrochloride on treatment with pyridine yields the *N*-ethoxycarbonyl derivative. Such a rearrangement of an acyl group from a sulphur to a nitrogen atom is well established for thioureas.[7]

2-Mercaptoglyoxaline on treatment with formaldehyde gave 1-hydroxymethyl-2-mercaptoglyoxaline.[8] 2-Mercapto-1-methylglyoxaline likewise gives a hydroxymethyl derivative which from chemical considerations would be expected to be 3(1)-hydroxymethyl-1(3)-methyl-2-thioglyoxalone. The spectrum of this compound shows strong absorption at 2630 Å which is explicable only in terms of the thione formula since the isomeric sulphide would be expected to show absorption typical of the *S*-alkylglyoxalines (cf. Table). It must therefore be concluded that the thione group in this particular environment is capable of absorbing strongly in the 2600 Å region.

Whether the absorption maximum at *ca.* 2600 Å (in ethanol) shown by non-acylated 2-mercaptoglyoxalines is due to the auxochromic effect of a thiol group on the glyoxaline chromophore thus appears to be questionable and the probability of its being due to the thione form emerges (cf. Koch [9]).

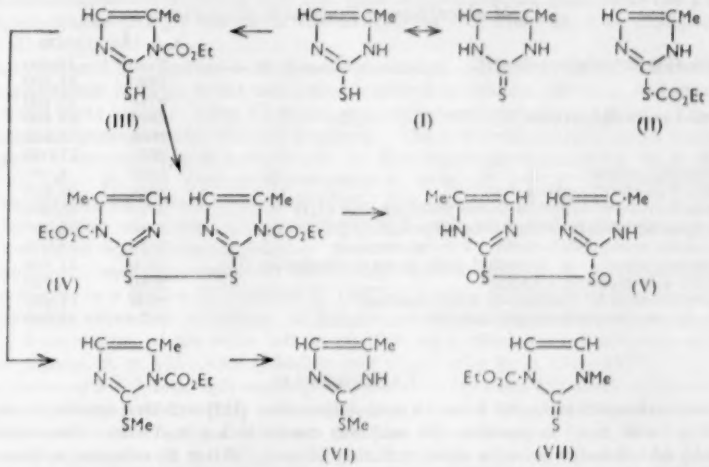
Spectroscopic investigation [10] of the thiol–thione tautomerism of 2-mercaptobenzothiazole and its derivatives [10] indicated that the thione form predominated in alcoholic solution since the *N*-methyl derivatives differed but little from the parent compound in their absorption, whereas the *S*-methyl derivatives absorbed at a lower wavelength with a great reduction in intensity. In alkaline solution the thione–thiol equilibrium was altered in favour of the " acidic " thiol form since the *S*-sodio-derivatives showed the absorption typical of the *S*-alkyl derivatives.

Analogous behaviour of the methyl derivatives of the 2-mercaptoglyoxalines has been observed (Table), *i.e.*, the absorption of the *S*-methyl derivative occurs at a slightly lower wavelength and with a much reduced intensity. It therefore appears that the absorption at 2600 Å in the 2-mercaptoglyoxalines is due mainly to contributions from the thione

[4] Dixon, *J.*, 1907, **912**.
[5] Anschutz and Schwickerath, *Annalen*, 1895, **284**, 99; Biltz and Krebs, *ibid.*, 1912, **391**, 203; Balaban and King, *J.*, 1927, 1858.
[6] Marckwald, *Ber.*, 1892, **25**, 2360.
[7] Dixon and co-workers, *J.*, 1920, **117**, 80, 720.
[8] Heath, Lawson, and Rimington, *J.*, 1951, 2218.
[9] Koch, *J.*, 1949, **401**.
[10] Morton and Stubbs, *J.*, 1939, 1321; Hassan and Hunter, *J.*, 1936, 1672.

form. This is supported by the work of Ettlinger [11] who on the basis of infrared absorption data considers that 2-mercapto-1-methylglyoxaline exists mainly in the thione form.

Accepting the view of Ettlinger and by analogy with 2-mercapto-4(5)-methylglyoxaline one would expect that acylation of 2-mercapto-1-methylglyoxaline would also take place at the nitrogen atom. The correctness of this view is shown in that the substance (VII), obtained by the action of ethyl chloroformate on the latter compound in pyridine, is non-basic and is also obtained by the action of pyridine on the hygroscopic *S*-ethoxycarbonyl-hydrochloride resulting from ethoxycarbonylation in non-basic media, as in the case of the 4(5)-methyl derivative above.



Grote's reagent,[12] used to distinguish between $\geq$C·SH and $\geq$C:S in organic sulphur compounds, gives a green colour with all mercaptoglyoxalines examined which have a potential thiol group and gives no colour with the sulphides. With the ethoxycarbonyl derivative of 2-mercapto-1-methylglyoxaline the colour produced is indigo, indicating the thione structure (VII). A number of other acylated derivatives of 2-mercapto-1-methylglyoxaline have been prepared with a view to their use as antithyroid substances. These, by analogy, have been designated as *N*-acyl derivatives.

In the case of 1(3)-ethoxycarbonyl-3(1)-methyl-2-thioglyoxalone (VII) the bathochromic shift in the absorption (Table) accompanying the introduction of the ethoxycarbonyl group may be attributed to the effect of this group on the absorption of the parent substance acting as the thione tautomer. For 1-ethoxycarbonyl-2-mercapto-4- or -5-methylglyoxaline (III) however a second explanation may be advanced. An examination of the infrared spectrum of 1-ethoxycarbonyl-2-mercapto-4- or -5-methylglyoxaline (III) in chloroform or Nujol shows that chelation of the mercapto- and ethoxycarbonyl groups takes place, since a splitting of the CO absorption band to give peaks at 5·65 and 5·74 $\mu$ is observed. The bathochromic shift from the 2600 to the 3050 Å region which occurs on the introduction of the *N*-ethoxycarbonyl group may, in this case, be alternatively attributed to this intramolecular hydrogen bonding (cf. Wiegand and Merkel;[13] Ferguson and Kelly [14]).

The acetyl derivative of 2-mercapto-4(5)methylglyoxaline (I) (Heath *et al.*[8]) was assigned the acetylthio-structure mainly on the basis of the lack of absorption in the 2600 Å region. Cook, Downer, and Heilbron,[15] however, tentatively assigned an *N*-acetyl structure to the monoacetyl derivative of 5-benzamido-2-mercaptoglyoxaline although again no

[11] Ettlinger, *J. Amer. Chem. Soc.*, 1950, **72**, 4699.
[12] Grote, *J. Biol. Chem.*, 1931, **93**, 25.
[13] Wiegand and Merkel, *Annalen*, 1947, **557**, 242.
[14] Ferguson and Kelly, *J. Amer. Chem. Soc.*, 1951, **73**, 3707.
[15] Cook, Downer, and Heilbron, *J.*, 1948, 1262.

absorption on the 2600 Å region was shown. In view of these conflicting conclusions, a dilute solution of the acetyl derivative of 2-mercapto-4(5)-methylglyoxaline in chloroform was titrated with iodine in the same solvent according to the procedure used by Werner [16] for thioureas. The rapid uptake of 1 equivalent of iodine, followed by a further slow uptake, presumably due to iodination of the ring, indicates the presence of a free thiol group. The compound in question must therefore be regarded as having the *N*-acetyl structure. The infrared spectrum of this compound substantiated such a formulation, the following bands being present : 2·9 μ (N–H monomeric), 3·12 μ (N–H associated), 6·02 μ (CH:CMe), 5·81 μ ($CH_3$·CO), 6·71 μ (NH·CS·N) and 3·70 μ (?) (trace of SH).

*Light absorption of 2-mercaptoglyoxalines.*

| | $\lambda_{max.}$ (Å) | ε | Solvent |
|---|---|---|---|
| 2-Mercapto-4(5)-methylglyoxaline (I) | 2570 | 15,000 | $H_2O$ |
| | 2630 | 14,700 | EtOH |
| | 2710 | 13,700 | $CHCl_3$ |
| 2-Mercapto-1-methylglyoxaline | 2510 | 14,400 | $H_2O$ |
| | 2600 | 13,600 | EtOH |
| | 2670 | 17,000 | $CHCl_3$ |
| 2-Methylthioglyoxaline | 2500 | 4,720 | EtOH |
| 4(5)-Methyl-2-methylthioglyoxaline (VI) | 2500 | 3,400 | EtOH |
| 1-Ethoxycarbonyl-2-mercapto-4(5)-methylglyoxaline (III) | 3090 | 15,600 | $CHCl_3$ |
| 1(3)-Ethoxycarbonyl-3(1)-methyl-2-thioglyoxalone (VII) | 3040 | 12,700 | $CHCl_3$ |
| 1(3)-Benzyloxycarbonyl-3(1)-methyl-2-thioglyoxalone | 3040 | 12,000 | $CHCl_3$ |
| Di-(1-ethoxycarbonyl-4- or -5-methyl-2-glyoxalinyl) disulphide (IV) | 2750 | 12,200 | $CHCl_3$ |
| 2-Benzylthio-4(5)-methylglyoxaline | 2510 | 5,020 | EtOH |
| 1(3)-Hydroxymethyl-3(1)-methyl-2-thioglyoxalone | 2630 | 14,400 | EtOH |
| 1 : 3-Di(hydroxymethyl)-2-thioglyoxalone | 2670 | 13,600 | EtOH |

## Experimental

*1-Ethoxycarbonyl-2-mercapto-4- or -5-methylglyoxaline* (III).—2-Mercapto-4(5)-methylglyoxaline (5·7 g., 0·05 mol.) in pyridine (20 ml.) was cooled in ice, and ethyl chloroformate (6 g., 0·055 mol.) added dropwise with stirring during 10 min. After 20 minutes, sodium hydrogen carbonate (4·2 g.) in water (30 ml.) was added, and the precipitate removed and recrystallised from water (7·2 g.). *1-Ethoxycarbonyl-2-mercapto-4- or -5-methylglyoxaline* had m. p. 121° (Found : C, 45·2; H, 5·5. $C_7H_{10}O_2N_2S$ requires C, 45·2; H, 5·4%). The product, sparingly soluble in water and dilute hydrochloric acid, dissolved readily in alkali. The introduction of a second ethoxycarbonyl group into 2-mercapto-4(5)-methylglyoxaline could not be effected by using excess of ethyl chloroformate.

*1-Ethoxycarbonyl-2-mercaptoglyoxaline.*—This *compound* prepared as above from 2-mercaptoglyoxaline (75% yield) had m. p. 119—20° (from ethyl acetate) (Found : C, 42·0; H, 4·8. $C_6H_8O_2N_2S$ requires C, 41·9; H, 4·7%).

*2-Ethoxycarbonylthio-4(5)-methylglyoxaline Picrate.*—To finely ground 2-mercapto-4(5)-methylglyoxaline (5·7 g.) in ethanol (100 ml.) was added ethyl chloroformate (6·0 g.), and the resulting suspension left overnight. Removal of the ethanol at room temperature gave a water-soluble, acidic yellow oil, which on addition of aqueous picric acid gave *2-ethoxycarbonylthio-4(5)-methylglyoxaline picrate* (3·0 g., 14·5%), m. p. 89—92° (Found : C, 37·9; H, 3·2. $C_{13}H_{13}O_9N_5S$ requires C, 37·6; H, 3·1%).

Addition of pyridine to an aqueous solution of the above oil gave 1-ethoxycarbonyl-2-mercapto-4- or 5-methylglyoxaline.

*Di-(1-ethoxycarbonyl-4- or -5-methyl-2-glyoxalinyl) Disulphide* (IV).—1-Ethoxycarbonyl-2-mercapto-4- or -5-methylglyoxaline (3·36 g., 0·018 mol.) was dissolved in acetic acid (40 ml.) and cooled to 0°. Concentrated nitric acid (2 ml.) was added with stirring during 30 min., followed by water (30 ml.) and then solid sodium carbonate to pH 7. The disulphide was filtered off and crystallised from aqueous ethanol to give yellow plates (3 g.), m. p. 138—139° (Found : C, 45·4; H, 4·8; N, 15·1; S, 17·3%; *M*, 376. $C_{14}H_{18}O_4N_4S_2$ requires C, 45·4; H, 4·9; N, 15·2; S, 17·3%; *M*, 370).

*Di-(1-ethoxycarbonyl-2-glyoxalinyl) disulphide*, yellow plates, m. p. 153° (from aqueous ethanol) (Found : C, 42·6; H, 4·1. $C_{12}H_{14}O_4N_4S_2$ requires C, 42·1; H, 4·1%), was similarly prepared.

[16] Werner, *J.*, 1912, 2168.

*Di-[4(5)-methyl-2-glyoxalinyl] Disulphoxide* (V).—Di-(1-ethoxycarbonyl-4- or -5-methyl-2-glyoxalinyl) disulphide (1·1 g., 0·0045 mol.) was added to 6N-sodium hydroxide (12 ml.) and left overnight. On neutralisation of the solution with 6N-hydrochloric acid a light yellow amorphous powder (0·8 g.), m. p. 250—254°, was precipitated. The product was initially soluble in ethanol but when kept it became insoluble in the usual organic solvents. Purification of the *disulphoxide* was effected by dissolving it in hydrochloric acid and reprecipitating it with sodium carbonate (Found: C, 37·1; H, 3·8. $C_8H_{10}O_2N_4S_2$ requires C, 37·2; H, 3·9%).

*2-Methylthio-4(5)-methylglyoxaline* (VI).—2-Mercapto-4(5)-methylglyoxaline (5·7 g., 0·05 mol.) was dissolved in ethanol, and methyl iodide (3·2 ml., 0·05 mol.) added. After 2 hr., the ethanol was removed under reduced pressure and aqueous potassium carbonate added to pH 10. The oil which separated slowly solidified at 0° and after recrystallisation from water the *2-methylthio-4(5)-methylglyoxaline* (3·3 g.) had m. p. 82—83° (Found: C, 47·0; H, 6·4. $C_5H_8N_2S$ requires C, 46·9; H, 6·3%).

*1-Ethoxycarbonyl-2-methylthio-4- or -5-methylglyoxaline.*—1-Ethoxycarbonyl-2-mercapto-4- or -5-methylglyoxaline (4·65 g., 0·025 mol.) was dissolved in ethanol (30 ml.), and methyl iodide (3·1 ml., 0·025 mol.) added. After 12 hr. aqueous potassium carbonate was added to pH 7 and the solutions evaporated under reduced pressure. The oily residue taken up in warm aqueous ethanol gave *1-ethoxycarbonyl-2-methylthio-4- or -5-methylglyoxaline*, needles, m. p. 46° (2·5 g.) (Found: C, 48·1; H, 5·8. $C_8H_{12}O_2N_2S$ requires C, 48·0; H, 5·9%). This substance (1·5 g.) was hydrolysed with 6N-sodium hydroxide (10 ml.) at room temperature (12 hr.). On acidification followed by addition of sodium carbonate to pH 9, 2-methylthio-4(5)-methylglyoxaline (above) separated as an oil which later solidified.

*2-Ethylthio-4(5)-methylglyoxaline.*—1-Ethoxycarbonyl-2-mercapto-4- or -5-methylglyoxaline (1 g.) was heated in a stream of nitrogen at 140° till carbon dioxide evolution ceased (0·5 hr.). Extraction with ether left a residue of 2-mercapto-4(5)-methylglyoxaline, m. p. 244—245° (45 mg.). Evaporation of the ether left an oil (500 mg.) which gave 2-ethylthio-4(5)-methylglyoxaline picrate, m. p. 135—136° (Balaban and King [8] give m. p. 136—137°).

*Halogenation of 2-Ethylthio-4(5)-methylglyoxaline.*—2-Ethylthio-4(5)-methylglyoxaline (2·8 g.) in chloroform (10 ml.) was treated with iodine (5 g.) for 0·5 hr. at room temperature. After being washed with water, the chloroform solution was evaporated and left *2-ethylthio-5(4)-iodo-4(5)-methylglyoxaline* (1 g., 19%), white needles (from aqueous ethanol), m. p. 166—167° (decomp.) (Found: C, 27·1; H, 3·6; N, 10·6. $C_6H_9N_2SI$ requires C, 26·9; H, 3·4; N, 10·5%). Substitution of bromine for iodine in the above reaction gave *5(4)-bromo-2-ethylthio-4(5)-methylglyoxaline*, m. p. 181—182° (decomp.) (Found: C, 32·8; H, 4·2; N, 12·7. $C_6H_9N_2SBr$ requires C, 32·6; H, 4·1; N, 12·7%).

*Acylation of 2-Mercapto-1-methylglyoxaline with Chloroformic Esters.*—2-Mercapto-1-methylglyoxaline, treated in pyridine with ethyl chloroformate as described above for 2-mercapto-4(5)-methylglyoxaline, gave *1(3)-ethoxycarbonyl-3(1)-methyl-2-thioglyoxaline*, m. p. 121—122° (from ethanol) (95% yield) (Found: C, 45·7; H, 5·4. $C_7H_{10}O_2N_2S$ requires C, 45·2; H, 5·4%). *2-Ethoxycarbonylthio-1-methylglyoxaline picrate*, m. p. 94—96° (decomp.), was obtained when the reaction was carried out in non-basic medium as for the 4(5)-methyl derivative (above) (Found: C, 37·4; H, 3·0. $C_{13}H_{13}O_9N_5S$ requires C, 37·6; H, 3·1%). Using methyl chloroformate in pyridine gave *1(3)-methoxycarbonyl-3(1)-methyl-2-thioglyoxalone*, m. p. 135° (from ethanol) (90% yield) (Found: C, 41·8; H, 4·4. $C_6H_8O_2N_2S$ requires C, 41·9; H, 4·7%). Benzyl chloroformate likewise gave *1(3)-benzyloxycarbonyl-3(1)-methyl-2-thioglyoxaline* (yield 10%), needles, m. p. 91—92°, from benzene–light petroleum, but a better yield (40%) was obtained by adding the benzyl chloroformate to a solution of the mercaptoglyoxaline in 4N-sodium hydroxide at 0° (Found: C, 58·3; H, 5·0. $C_{12}H_{12}O_2N_2S$ requires C, 58·1; H, 4·8%).

*1(3)-Hippuroyl-3(1)-methyl-2-thioglyoxaline.*—To 2-mercapto-1-methylglyoxaline (0·01 mol.), dissolved in 2-picoline (10 ml.), 2-phenyloxazol-5-one (0·01 mol.) was slowly added. After 24 hr. the solution was poured into ice-water; the precipitated *1(3)-hippuroyl-3(1)-methyl-2-thioglyoxalone*, purified by recrystallisation from ethyl acetate, had m. p. 175° (Found: C, 56·4; H, 5·0; N, 15·3. $C_{12}H_{12}O_2N_2S$ requires C, 56·9; H, 4·7; N, 15·3%).

*2-Benzoylthio-1-methylglyoxaline Hydrochloride.*—2-Mercapto-1-methylglyoxaline (0·01 mol.) was heated in benzene (25 ml.) with benzoyl chloride (0·01 mol.) at 100°. The precipitated *hydrochloride*, recrystallised from ethanol, had m. p. 183° (Found: C, 51·7; H, 4·1. $C_{11}H_{11}ON_2SCl$ requires C, 51·9; H, 4·3%).

*3(1)-Benzoyl-1(3)-methyl-2-thioglyoxalone.*—When the benzoylation of 1-methyl-2-mercaptoglyoxaline was carried out in pyridine and a few drops of ethanol were added, yellow needles of the *glyoxalone*, m. p. 102° (from ethanol), were obtained (Found: C, 59·7; H, 4·4. $C_{11}H_{10}ON_2S$

requires C, 60·5; H, 4·6%). This was also obtained by treatment of the above hydrochloride with aqueous sodium hydrogen carbonate.

*2-Ethoxycarbonylmethylthio-1-methylglyoxaline Hydrochloride.*—Ethyl chloroacetate (0·01 mol.) was heated with 2-mercapto-1-methylglyoxaline (0·01 mol.) in ethanol (10 ml.) at 100° for 30 min. The hydrochloride was precipitated by ether, and recrystallised from ethanol–ether as prisms, m. p. 99° (Found: C, 40·8; H, 5·6. $C_8H_{13}O_2N_2SCl$ requires C, 40·6; H, 5·5%). Using chloroacetic acid instead of the ester (above) gave *2-carboxymethylthio-1-methylglyoxaline hydrochloride*, m. p. 162° (from ethanol) (Found: C, 35·1; H, 4·6. $C_6H_9O_2N_2SCl$ requires C, 34·5; H, 4·3%).

*1(3)-Hydroxymethyl-3(1)-methyl-2-mercaptoglyoxaline.*—2-Mercapto-1-methylglyoxaline (1 g.) and neutralised formaldehyde solution (6 ml. of 40%) were heated together on the water-bath for 1 hr. Ethanol (20 ml.) was then added and heating continued for a further 1 hr. The deposited crystals (0·6 g.) of the *hydroxymethyl derivative*, recrystallised from alcohol, had m. p. 115—118° (Found: C, 41·9; H, 5·7; N, 19·7. $C_5H_8ON_2S$ requires C, 41·7; H, 5·6; N, 19·4%).

*4- or 5-Ethoxycarbonyl-1-hydroxymethyl-2-mercaptoglyoxaline.*—4(5)-Ethoxycarbonyl-2-mercaptoglyoxaline was treated with formaldehyde as above, to give *4- or 5-ethoxycarbonyl-1-hydroxymethyl-2-mercaptoglyoxaline* (1·8 g., 73%), m. p. 138—139° (from water) (Found: C, 41·5; H, 5·0; N, 13·9. $C_7H_{10}O_3N_2S$ requires C, 41·6; H, 5·0; N, 13·9%).

*1 : 3-Di(hydroxymethyl)-2-thioglyoxalone.*—2-Mercaptoglyoxaline (0·5 g.) was heated on the steam-bath with neutralised aqueous formaldehyde (5 ml. of 40%) for 0·5 hr. The solution was then evaporated under reduced pressure and the remaining *glyoxalone* crystallised from ethanol as prisms, m. p. 129° (Found: C, 37·5; H, 4·7; N, 17·4. $C_5H_8O_3N_2S$ requires C, 37·5; H, 5·0; N, 17·5%).

We thank Dr. J. E. Page of Glaxo Laboratories for carrying out the infrared measurements.

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## 231. *The Reactions of Anthracene and 9-Methylanthracene with the Free Radicals derived from Di-*tert.*-butyl Peroxide.*

By A. L. J. Beckwith and William A. Waters.

The thermal decomposition of di-*tert.*-butyl peroxide in boiling chlorobenzene or *o*-dichlorobenzene has been used as a source of methyl and *tert.*-butoxy-radicals, and the reactions of these radicals with some polycyclic aromatic hydrocarbons have been investigated. Little significant reaction occurs with chlorobenzene, naphthalene, phenanthrene, or pyrene, but anthracene gives both 9-methylanthracene (IV) and 9 : 9′ : 10 : 10′-tetrahydro-10 : 10′-dimethyl-9 : 9′-dianthryl (III), the formation of the latter being favoured by low temperatures.

9-Methylanthracene is more reactive than anthracene. It yields 9 : 10-dimethylanthracene by nuclear substitution, 1 : 2-di-9′-anthrylethane by dehydrogenation followed by dimerisation, and smaller quantities of 9-ethyl-, 9-ethyl-10-methyl-, and 9 : 10-diethyl-anthracene, and 1-9′-anthryl-2-(10-methyl-9-anthryl)ethane (XII) by further reactions of the same type.

Since these secondary reactions of anthracene occur so readily, investigations of the initial products of the reaction between anthracene and methyl radicals do not give a correct measure of the ease of this reaction.

Some years ago it was shown by Roitt and Waters [1] that when certain polycyclic aromatic hydrocarbons such as anthracene, 1 : 2-benzanthracene, and 3 : 4-benzopyrene were allowed to react with the homolytic decomposition products of benzoyl peroxide they were substituted in their active *meso*-positions by benzoate groups. In contrast, benzene and many of its less active derivatives are phenylated when subjected to the same reaction.[2]

[1] Roitt and Waters, *J.*, 1952, 2695.
[2] Augood, Hey, and Williams, *J.*, 1952, 2094.

As a continuation of this work we have investigated the reaction between the free radicals formed by the thermal decomposition of di-*tert.*-butyl peroxide,[3] and chlorobenzene, naphthalene, phenanthrene, pyrene, anthracene, and 9-methylanthracene, but only with anthracene and its methyl derivative could we obtain appreciable yields of distinctive reaction products. All the other hydrocarbons were recovered unchanged. This high reactivity of anthracene and its derivatives towards free radicals is in accord with current theories of free valency,[4] and with the analysis by Levy and Szwarc [5] of the gaseous products of reactions between acetyl peroxide and aromatic hydrocarbons.

Our results, which support the widely accepted view that free radicals react first by addition to the carbon atoms of aromatic molecules, indicate that with the radicals $CH_3$· and $(CH_3)_3C{\cdot}O$· formed from di-*tert.*-butyl peroxide, the primary aromatic reaction product is certainly equal to, and usually greater than, the starting material in reactivity. Hence a number of secondary free-radical reactions can occur and the percentage yields of the various products actually isolated give little indication of the overall extent of the primary reaction.

The first experiments were conducted in refluxing chlorobenzene which, we had hoped, would prove quite inert to decomposing di-*tert.*-butyl peroxide. However preliminary work showed that this was not so. When chlorobenzene and the peroxide were refluxed together for three days and the volatile products collected, a considerable amount of *tert.*-butanol was obtained, indicating that *tert.*-butoxy-radicals had attacked the liquid mixture. No pure products such as chlorotoluenes or dichlorodiphenyls could be isolated but an appreciable amount of involatile resin was obtained. This residue, which probably resulted from radical polymerisation, greatly increased the difficulty of isolating the crystalline products from later experiments.

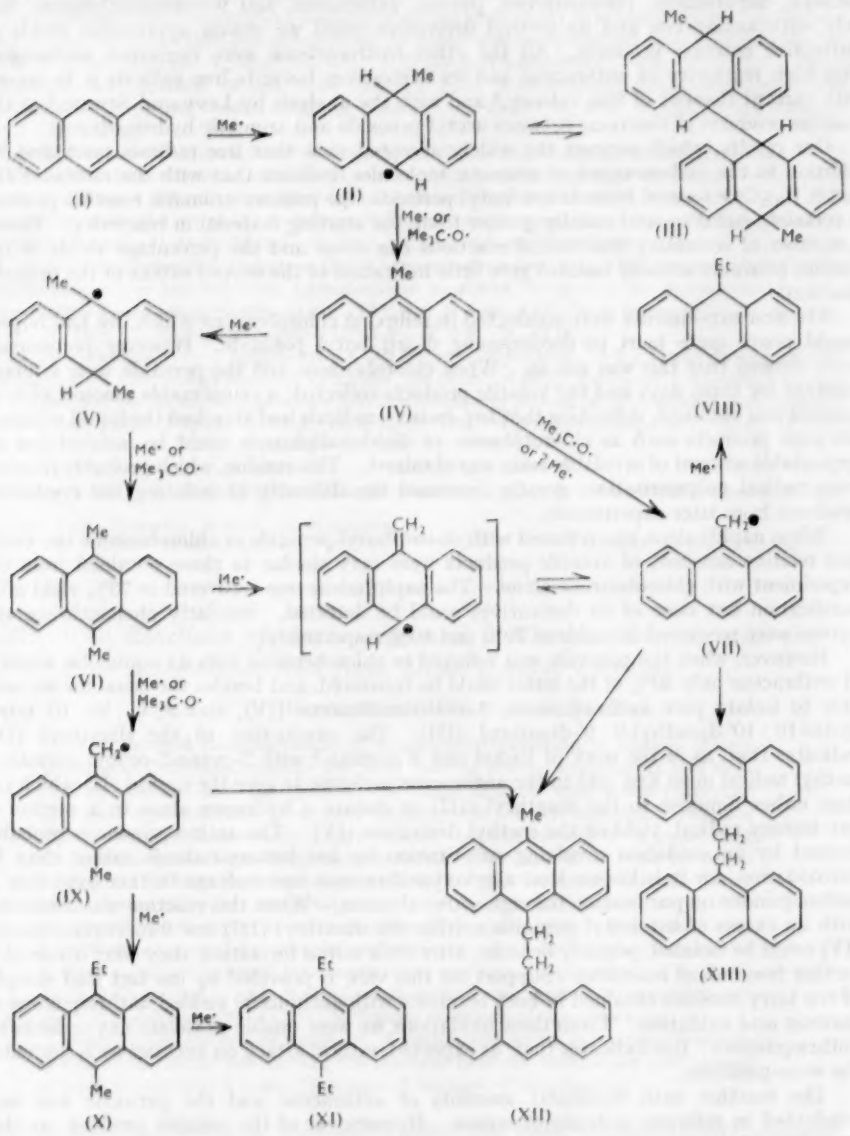
When naphthalene was refluxed with di-*tert.*-butyl peroxide in chlorobenzene the yields and relative amounts of volatile products were very similar to those obtained from the experiment with chlorobenzene alone. The naphthalene was recovered in 75% yield after purification but none of its derivatives could be detected. Similarly phenanthrene and pyrene were recovered in yields of 70% and 80% respectively.

However, when the peroxide was refluxed in chlorobenzene with an equimolar amount of anthracene only 40% of the latter could be recovered, and besides the usual tar we were able to isolate pure anthraquinone, 9-methylanthracene (IV), and 9 : 9′ : 10 : 10′-tetrahydro-10 : 10′-dimethyl-9 : 9′-dianthryl (III). The production of the dianthryl (III) indicates that, as in the work of Bickel and Kooyman [6] with 2-cyano-2-propyl radicals, a methyl radical must first add to the anthracene molecule to give the radical (II) which can then either dimerise to the dianthryl (III) or donate a hydrogen atom to a methyl or *tert.*-butoxy-radical, yielding the methyl derivative (IV). The anthraquinone is probably formed by an oxidation involving substitution by *tert.*-butoxy-radicals rather than by autoxidation, for it is known that alkyloxyanthracenes can undergo further oxidation to anthraquinone on purification through active alumina. When this reaction was conducted with an excess of *tert.*-butyl peroxide neither the dianthryl (III) nor 9-methylanthracene (IV) could be isolated, possibly because, after their initial formation, they were involved in further free-radical reactions. Support for this view is provided by the fact that samples of the tarry residues obtained in both reactions with anthracene yielded anthraquinone on chromic acid oxidation. From these oxidations we were unable to isolate any substituted anthraquinones; this indicates that, as expected, radical attack on anthracene is limited to the *meso*-positions.

The reaction with equimolar amounts of anthracene and the peroxide was next conducted in refluxing *o*-dichlorobenzene. By removal of the volatile products as they were formed the temperature in the boiling mixture was kept at 155—165°. The reaction was then rapid and gave increased yields of anthraquinone and 9-methylanthracene, but the dimeric product (III) was not isolated. This was probably due to the fact that high

[3] Raley, Rust, and Vaughan, *J. Amer. Chem. Soc.*, 1948, **70**, 88.
[4] Coulson, *J.*, 1955, 1435.
[5] Levy and Szwarc, *J. Amer. Chem. Soc.*, 1955, **77**, 1949.

temperatures favour the reverse reaction (III ⟶ II) as shown by the work of Bickel and Kooyman.[6]




A low-temperature experiment was carried out in the absence of a solvent but only unchanged starting material was recovered, probably because of the insolubility of anthracene in the peroxide.

Attention may be drawn to the fact that none of these reactions involving anthracene

[6] Bickel and Kooyman, *Rec. Trav. chim.*, 1952, **71**, 1137.

led to the formation of 9 : 9′-dianthryl : this indicates that the direct extraction of hydrogen from the anthracene molecule does not occur.

Since in the above experiments there was some evidence of further reaction, to give more highly substituted anthracenes, the thermal decomposition of di-*tert.*-butyl peroxide in the presence of 9-methylanthracene was next examined. Reaction in refluxing chlorobenzene gave, besides unchanged starting material, 1 : 2-di-9′-anthrylethane (XIII) and its polymer (probably identical with that reported by Roitt and Waters [1]), 9 : 10-dimethyl- (VI), 9-ethyl-10-methyl- (X), and 9-ethyl-anthracene (VIII). These substances are probably formed by the routes shown in the annexed scheme. Further information on the mode of reaction was provided by heating the peroxide and 9-methylanthracene without solvent on a bath of boiling toluene. At this lower temperature the hydrocarbon was converted into the coupling product (XIII) and its polymer, and no substitution product was isolated. The only volatile product was *tert.*-butyl alcohol, indicating that under mild conditions the *tert.*-butoxy-radical does not decompose rapidly, and that it is reactive enough to abstract hydrogen from the side chain of 9-methylanthracene. On the other hand, on reaction in boiling *o*-dichlorobenzene there was a notable increase in the amounts of substitution products. This may indicate that at high temperatures the methyl radical is the more important reactant and that methyl rather than *tert.*-butoxy is the radical which easily combines with the anthracene nucleus.

The production of 9 : 10-diethylanthracene (XI) in this high-temperature reaction was unexpected, since the only available route is from 9-methyl-10-ethylanthracene by dehydrogenation and methylation of the 9-methyl group, and it would be expected that the hydrogen atoms on this group would be less reactive than those in the α-position of the ethyl group. However a model showed that steric hindrance due to the side rings may be a contributing factor in deciding the course of hydrogen abstraction. Another point of interest was that 9-ethyl-10-methylanthracene was formed from the radical (IX) in much higher yield than was 1-9′-anthryl-2-(10-methyl-9-anthryl)ethane (XII), and the dimer, 1 : 2-di-(10-methyl-9-anthryl)ethane was not formed in detectable amount. This indicates that the stationary concentrations in the reaction mixture of the radicals involved in the production of the above compounds were $\cdot CH_3 > (VII) > (IX)$.

All of the products isolated from these reactions were identified by comparison of melting points and infrared and ultraviolet spectra with those of the authentic materials. In one case 1 : 2-di-(10-methyl-9-anthryl)ethane the compound prepared by us differed considerably from that previously described.[7] We believe that the material described here is the authentic compound since it has the ultraviolet absorption typical of anthracene derivatives, and that the substance obtained by Badger and Pearce [7] was its dihydro-derivative which we have also prepared by another method.

## Experimental

*Materials.*—Di-*tert.*-butyl peroxide was purified by distilling the commercial material under reduced pressure. The fraction of b. p. 50—52°/90 mm. was collected and used immediately.

Naphthalene, phenanthrene, and pyrene were recrystallised to satisfactory m. p.

Anthracene was purified by two distillations with ethylene glycol.

9-Methylanthracene was first prepared from 9-anthraldehyde [8] by the Huang-Minlon procedure [9] but later experiments showed the following variation of the method of Krollpfeiffer and Bronscheid [10] to be more satisfactory. A suspension of anthrone (16·0 g.) in ether (80 ml.) was added in small portions to a vigorously stirred Grignard solution prepared from methyl iodide (36 g.) and magnesium (6·0 g.) in 60 ml. of ether. After the addition (20 min.) the mixture was refluxed for 2·5 hr. and the excess of reagent was then destroyed by the addition of 15% sulphuric acid. The ether layer was collected and after removal of the solvent the residue was chromatographed on alumina (250 g.; activity I). Elution with 1 : 1 light petroleum–benzene

[7] Badger and Pearce, *J.*, 1950, 2314.
[8] *Org. Synth.*, 1940, **20**, 11.
[9] Huang-Minlon, *J. Amer. Chem. Soc.*, 1949, **71**, 3301.
[10] Krollpfeiffer and Bronscheid, *Ber.*, 1923, **56**, 1617.

gave 9-methylanthracene which crystallised from methanol as very pale yellow plates (13·2 g., 84%), m. p. 80°.

Chromatographic alumina was prepared by washing the commercial product with warm 10% hydrochloric acid, then large amounts of water, and finally methanol. The neutral material was reactivated and standardised by the method of Brockmann and Schodder.[11] The light petroleum used in chromatographic separations had b. p. 40—60°.

*Reactions of Chlorobenzene with Di-*tert.*-butyl Peroxide.*—A mixture of chlorobenzene (150 ml.) and the peroxide (15·0 g.) was refluxed, away from direct light, for 70 hr. The volatile products were separated by fractional distillation through a helix-packed column. The first fraction (4·1 g.), b. p. 56—60°, proved to be acetone (2 : 4-dinitrophenylhydrazone, m. p. 127°). The second fraction (4·8 g.), b. p. 78—82°, crystallised and was *tert.*-butyl alcohol. No unchanged di-*tert.*-butyl peroxide was recovered. After removal of these low-boiling fractions the solvent was distilled off under reduced pressure, leaving a black tar (4·6 g.) which was chromatographed on alumina (activity II). No crystalline products were obtained. Most of the fractions were oils with strong odours similar to those of the chlorodiphenyls, but there was also eluted an amorphous yellow powder which gave a weak positive test for halogen and slowly sintered when heated.

*Reaction of Naphthalene with Di-*tert.*-butyl Peroxide in Chlorobenzene.*—Di-*tert.*-butyl peroxide (20 g.) was added slowly to a boiling solution of naphthalene (16·0 g.) in chlorobenzene (250 ml.), and the mixture was refluxed for 70 hr. Separation of the low-boiling fractions by fractional distillation yielded acetone (5·6 g.) and *tert.*-butyl alcohol (5·7 g.). After removal of the solvent (vacuum) the tarry residue was steam-distilled, to give unchanged naphthalene (12·0 g. after crystallisation). The residue (6·4 g.) was chromatographed on alumina (250 g.; activity II) but again only intractable oils and tars, very similar in physical properties to those obtained in the previous experiment, were eluted.

*Reaction of Anthracene with Di-*tert.*-butyl Peroxide in Chlorobenzene.*—Equimolar quantities of anthracene (18·0 g.) and the peroxide (15·0 g.) were refluxed for 70 hr. in chlorobenzene (250 ml.), and the low-boiling products were removed by fractional distillation. Acetone (4·7 g.) and *tert.*-butyl alcohol (6·9 g.) were obtained. Distillation was continued until 160 ml. of solvent had been collected and the remaining solution was set aside at 0°. Anthracene (3·5 g.), m. p. and mixed m. p. 217°, was precipitated. The mother-liquors were evaporated under reduced pressure, leaving a dark brown oil (15·6 g.). This was refluxed thrice with 150 ml. portions of ethanol which dissolved most of the material, leaving 1·25 g. of colourless powder, m. p. 242°, which, after repeated crystallisation from chloroform was obtained as colourless prisms, m. p. 264° (Found: C, 92·4; H, 6·65%). Comparison of the ultraviolet and infrared spectra showed it to be 9 : 9′ : 10 : 10′-tetrahydro-10 : 10′-dimethyl-9 : 9′-dianthryl. Sulphur dehydrogenation at 230° converted it into 9-methylanthracene (70%), m. p. and mixed m. p. 80°. Evaporation of the mother-liquors left 14·0 g. of brown oil which when refluxed with light petroleum (100 ml.) left 1·25 g. of a mixture of anthracene and anthraquinone. Chromatography of the petroleum solution on alumina (activity II) yielded anthracene, 9-methylanthracene, m. p. 79° (partly isolated as the picrate, m. p. and mixed m. p. 137°), anthraquinone, and the above tetrahydrodimethyldianthryl; total yields were: anthracene 7·25 g. (40%); 9 : 9′ : 10 : 10′-tetrahydro-10 : 10′-dimethyl-9 : 9′-dianthryl, 1·37 g. (7%); anthraquinone, 0·66 g. (3%); 9-methylanthracene, 1·44 g. (7·5%).

*Reaction of Anthracene with Excess of Peroxide in Chlorobenzene.*—Anthracene and 2 molar proportions of *tert.*-butyl peroxide were refluxed in chlorobenzene under a stream of air for 70 hr. Apparently the low-boiling products were blown through the condenser as no acetone or *tert.*-butyl alcohol was obtained. Removal of the solvent left a dark red viscous oil which was divided into fractions by treatment with hot light petroleum. The petroleum-insoluble fraction, on chromatography, yielded anthraquinone (7%) and various red and yellow oils which, when oxidised with chromic acid in acetic acid solution, all yielded anthraquinone. The petroleum-soluble fraction gave unchanged anthracene (6%) and oils which could not be crystallised.

*Reaction of Anthracene with Di-*tert.*-butyl Peroxide.*—(*a*) *In o-dichlorobenzene.* Anthracene (9 g.), the peroxide (9 g.), and *o*-dichlorobenzene (100 ml.) were refluxed in an atmosphere of nitrogen. The reflux condenser was packed with glass helices and surrounded with a jacket of boiling benzene which allowed volatile products to be distilled off as they were formed. By this means the temperature of the reaction mixture was maintained at 155—165°. After 3 hr. the solvent was removed at 100° under reduced pressure, leaving a brown oil (17 g.) which was

[11] Brockman and Schodder, *Ber.*, 1941, **74**, 73.

divided into petroleum-soluble and -insoluble fractions and chromatographically separated in the usual way. The total yields of products were: anthracene, 0·8 g. (9%); 9-methylanthracene, 2·6 g. (27%); anthraquinone, 2·2 g. (21%).

(*b*) *Without solvent.* When anthracene (5 g.) and di-*tert.*-butyl peroxide (6 g.) were heated together without solvent under nitrogen in a bath of boiling toluene for 5 days, only unchanged anthracene (4·5 g.) was recovered.

*Reaction of 9-Methylanthracene with Di-*tert.*-butyl Peroxide.*—(a) *In chlorobenzene.* The hydrocarbon (13·6 g.), the peroxide (12·0 g.), and chlorobenzene (90 ml.) were refluxed under nitrogen for 70 hr. Low-boiling products and solvent (total 40 ml.) were distilled off through a fractionating column, and the remaining liquor was set aside overnight at 0°. After separation of the colourless crystalline precipitate (A) (0·84 g.) the solution was taken to dryness (vacuum) under nitrogen. The residue was extracted four times with boiling light petroleum (total 300 ml.) which removed 7·0 g. and left a red oil that was freely soluble in benzene. When cooled, the petroleum extract deposited a yellow amorphous precipitate (0·58 g.), and similar material (0·32 g.) was obtained by concentration of the benzene solution. After repeated crystallisation from toluene this material was identified as 1 : 2-di-9′-anthrylethane.

Product (A), after two recrystallisations from toluene, formed needles, m. p. 310° (Found: C, 93·8; H, 6·0. $C_{28}H_{22}$ requires C, 94·2; H, 5·8%). Light absorption in $CHCl_3$ showed a weak maximum at 2725 Å (no absorption between 3000 and 4000 Å). After it had been heated in an evacuated tube at 310—312° until all the material had melted and then cooled, the substance was crystallised from toluene, forming yellow crystals (yield 92%) of 1 : 2-di-9′-anthrylethane (m. p. and mixed m. p. 310°). Substance (A) must therefore be a *polymer* of this hydrocarbon.

The material soluble in light petroleum was separated chromatographically and the successive fractions were further separated by repeated crystallisation from ethanol, methanol, and light petroleum and also by conversion into picrates. There were obtained in this way in all: 9-methylanthracene, 1·7 g. (12·5%) (picrate m. p. 137°); 1 : 2-di-9′-anthrylethane, 1·81 g. (13·5%); polymer of 1 : 2-di-9′-anthrylethane, 2·21 g. (16·5%); 9-ethyl-10-methylanthracene, 55 mg.; 9 : 10-dimethylanthracene, 0·80 g. (5·5%); 9-ethylanthracene, 20 mg.

(b) *Without solvent.* The hydrocarbon (9·8 g.) and the peroxide (11·0 g.) were heated, under reflux, in a bath of boiling toluene. After 5 days a small amount of the solvent was distilled off through a helix-packed column. The temperature of the distilling vapours rose immediately to 80° (b. p. of *tert.*-butyl alcohol), and no acetone was obtained. The mixture was then cooled and diluted with acetone, and its components were separated as described above. It gave: 9-methylanthracene, 4·5 g. (46%); 1 : 2-di-9′-anthrylethane and its polymer, 4·19 g. (42%).

(c) *In o-dichlorobenzene.* 9-Methylanthracene (10·5 g.) and the peroxide (12·0 g.) were refluxed in *o*-dichlorobenzene (90 ml.) under nitrogen. The volatile products were allowed to escape with the nitrogen stream through a fractionating column and by this means the temperature of the reacting mixture was maintained at 150—155°. After 48 hr. the solvent was removed in a vacuum and the residue was separated as described above into: 1 : 2-di-9′-anthrylethane, 0·75 g. (7%); its polymer, 0·95 g. (9%); 9 : 10-dimethylanthracene, 2·15 g. (20%); 9-ethyl-10-methylanthracene, 1·05 g. (9%); 9 : 10-diethylanthracene, 50 mg.; 1-9′-anthryl-2-(10-methyl-9-anthryl)ethane, 45 mg.

*Reaction of Pyrene and of Phenanthrene with Di-*tert.*-butyl Peroxide.*—When phenanthrene (10 g.) and the peroxide (13 g.) were refluxed in *o*-dichlorobenzene for 3 hr. in the apparatus which allowed the volatile products to escape and the mixture was worked up in the usual way the only pure substance isolated was unchanged phenanthrene (8·6 g.). When pyrene (10 g.) was similarly treated with the peroxide (9 g.), 8·1 g. of unchanged pyrene were recovered.

*Reference Materials.*—(i) 9-Ethylanthracene, prepared from anthrone and ethylmagnesium iodide, crystallised from ethanol in fluorescent plates, m. p. 58° (yield 87%); the dark red picrate had m. p. 120°. (ii) 9-Ethyl-10-methylanthracene, prepared from 10-methylanthrone in 92% yield, crystallised from ethanol in yellow needles, m. p. 144°; the picrate had m. p. 137°. (iii) 9 : 10-Diethylanthracene: a Grignard solution was prepared from ethyl iodide (9·8 g.) and magnesium (1·5 g.) in ether (50 ml.), cooled in ice, and treated, with vigorous stirring, with crystallised anthraquinone (3·0 g.). The stirred mixture was gradually warmed, then refluxed for 2 hr. and eventually decomposed with ammonium chloride solution. There was obtained a solid yellow mixture of crude isomeric forms of 9 : 10-diethyl-9 : 10-dihydro-9 : 10-dihydroxyanthracene. This mixture * was warmed in acetic acid (30 ml.) with phenylhydrazine (6·0 g.). After the initial vigorous evolution of nitrogen the solution was refluxed for 30 min., cooled, and

* We thank Dr. K. J. Clark for making available to us the following details.

poured into dilute hydrochloric acid. The precipitate was chromatographed in 1 : 1 light petroleum–benzene on alumina (150 g.; activity II). The first fraction eluted was 9 : 10-diethylanthracene which crystallised from ethanol as pale yellow needles (2·6 g., 77%), m. p. 148° (black picrate, m. p. 130°).

9 : 9′ : 10 : 10′-*Tetrahydro*-10 : 10′-*dimethyl*-9 : 9′-*dianthryl*. 10 : 10′-Dimethyl-9 : 9′-dianthryl (0·50 g.), prepared by the reduction of 10-methylanthrone with zinc dust and ammonia,[12] was gently refluxed in pentyl alcohol (30 ml.) while sodium (2·0 g.) was added in small pieces during 2 hr., then the solution was diluted with methanol, cooled, and poured into water. The precipitate was filtered off and washed with water and thrice with ether. Crystallisation from chloroform gave prisms (180 mg.) of trans-trans ?-9 : 9′ : 10 : 10′-*tetrahydro*-10 : 10′-*dimethyl*-9 : 9′-*dianthryl*, m. p. 264° (Found : C, 93·0; H, 6·8. $C_{30}H_{26}$ requires C, 93·2; H, 6·8%). Light absorption in $CHCl_3$ : max. at 2650 Å (ε 1750). The ether washings were evaporated to a crystalline mixture (130 mg.), possibly consisting of stereoisomers of the tetrahydro-compound.

1 : 2-*Di*-9′-*anthrylethane*. This was prepared by reduction of 10-methyleneanthrone with zinc and hydrochloric acid in acetic acid ;[12,1] it crystallised from toluene as pale yellow needles, m. p. 310°. Light absorption in $CHCl_3$ : max. at 2550 (ε 22,500), 3400 (ε 439), 3560 (ε 1180), 3750 (ε 2400), and 3980 Å (ε 3050).

2-9′-*Anthrylethyl toluene*-p-*sulphonate*. To a solution of butyl-lithium [from *n*-butyl chloride (2·5 g.) and lithium (0·40 g.) in ether (50 ml.)] 9-bromoanthracene [13] (5·0 g.) was rapidly added and the solution was stirred at room temperature for 10 min., then treated during 10 min. at <0° with a cooled solution of ethylene oxide (3·0 g.) in ether (20 ml.). Stirring was continued for 30 min. with cooling and 15 min. at the b. p. Water was added, the ether layer was washed and dried, and the solvent was removed under reduced pressure. The residue (3·7 g.), dissolved in dry pyridine (15 ml.), was cooled to 0°. With continued cooling a solution of toluene-*p*-sulphonyl chloride (6·0 g.) in pyridine (10 ml.) was slowly added. After 24 hr. at room temperature the excess of the chloride was destroyed by water (2 ml.) and the mixture was then poured into iced water. The precipitated *ester* solidified and was washed with dilute acid and water and crystallised from ethanol as fluorescent plates (5·1 g., 69%), m. p. 122° (Found : C, 72·8; H, 5·6; S, 8·7. $C_{23}H_{20}O_3S$ requires C, 73·4; H, 5·35; S, 8·5%). Light absorption in EtOH : max. at 3340 (ε 2685), 3500 (ε 5390), 3660 (ε 8100), and 3880 Å (ε 7960).

2-9′-*Anthrylethyl iodide*. A solution of the foregoing ester (3·6 g.) and dry sodium iodide (3·0 g.) in acetone (30 ml.; freshly distilled from anhydrous copper sulphate) was refluxed for 18 hr., cooled, and poured into water. The oily precipitate was extracted with ether and was washed and dried. After removal of the solvent the residue was chromatographed on alumina (150 g.; activity III). Light petroleum eluted the *iodide* (3·0 g., 94%) which crystallised from light petroleum or methanol as pale yellow, fluorescent needles, m. p. 112° (Found : C, 57·85; H, 4·15; I, 37·8. $C_{16}H_{13}I$ requires C, 57·85; H, 3·9; I, 38·2%). Light absorption in EtOH : max. at 2480 (ε 106,500), 2560 (ε 204,000), 3200 (ε 2030), 3350 (ε 4220), 3510 (ε 8750), 3700 (ε 13,500), and 3900 Å (ε 13,380).

1-9′-*Anthryl*-2-(10-*methyl*-9-*anthryl*)*ethane*. Reaction between the above-mentioned iodide (0·78 g.) in dry ether (20 ml.) with magnesium turnings (60 mg.) was initiated by methyl iodide and iodine and was completed by refluxing for 2 hr. A solution of 10-methylanthrone (0·5 g.) in ether (20 ml.) was added dropwise with stirring and the mixture was refluxed for 1 hr., then cooled and neutralised with dilute hydrochloric acid. The ether was washed and dried and the solvent was removed. The residue was chromatographed on alumina (50 g.; activity I). Light petroleum eluted unchanged iodide (0·41 g.) and 1 : 1 light petroleum–benzene eluted the required *hydrocarbon* (0·36 g.) which crystallised from acetone as pale yellow needles, m. p. 236° (Found : C, 93·6; H, 6·3. $C_{31}H_{24}$ requires C, 93·9; H, 6·1%). Light absorption in $CHCl_3$ : max. at 2650 (ε 71,000), 3850 (ε 12,600), and 4050 Å (ε 13,100).

9-*Iodomethyl*-10-*methylanthracene*. Finely powdered anthraquinone (6·0 g.) was added rapidly to an ice-cold Grignard solution prepared from methyl iodide (18 g.) and magnesium (3·0 g.) in ether (80 ml.). Then the mixture was gently warmed and finally refluxed, with stirring, for 3 hr. The resultant yellow suspension of the magnesium salt of 9 : 10-dimethyl-9 : 10-dihydro-9 : 10-dihydroxyanthracene was converted into the iodomethyl compound by the method of Badger and Pearce.[7] The yield of unstable yellow needles was 7·4 g. (77%).

1 : 2-*Di*-(10-*methyl*-9-*anthryl*)*ethane*. Reaction between the above-mentioned iodomethyl compound (1·5 g.) and magnesium (0·12 g.) in benzene (15 ml.) and ether (25 ml.), initiated as above, was completed by refluxing, with stirring, for 30 min. Anhydrous cobalt chloride

[12] Barnett and Matthews, *Ber.*, 1926, **59**, 767.
[13] Barnett and Cook, *J.*, 1924, 1084.

(0·6 g.) was added and the mixture was stirred and refluxed for a further hour. After cooling, dilute hydrochloric acid was added and the yellow crystalline precipitate was collected, washed with ether, and crystallised from benzene. 1 : 2-*Di*-(10-*methyl*-9-*anthryl*)*ethane* formed bright yellow needles (0·76 g., 82%), m. p. 272° (Found : C, 93·6; H, 6·4. $C_{38}H_{26}$ requires C, 93·6; H, 6·4%). Light absorption in $CHCl_3$ : max. at 2650 (ε 49,800), 3500 (ε 2500), 3600 (ε 3945), 3850 (ε 7000), and 4100 Å (ε 10,000).

*Reduction of* 1 : 2-*Di*-(10-*methyl*-9-*anthryl*)*ethane*. The hydrocarbon (0·50 g.) was refluxed in butan-1-ol (20 ml.) while sodium (1·0 g.) was added in small pieces. After 30 min. all the sodium and hydrocarbon had dissolved to give a colourless solution, which was cooled and diluted with methanol (20 ml.). The precipitate of the *dihydro-compound* crystallised from benzene–alcohol as cubes (0·43 g.), m. p. 304° (Found : C, 92·9, 39·0; H, 6·7, 6·5. $C_{38}H_{28}$ requires C, 93·2; H, 6·8%). Light absorption in $CHCl_3$ : max. at 2650 (ε 1960), 2750 (ε 2460), and 3100 Å (ε 317).

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## 232. *Stabilities of Complex Compounds of Metals. The System Di(tri-*n*-butylphosphine)dichloropalladium(II)–*n*-Octylamine.*

By B. MEDDINGS and A. R. BURKIN.

The successive equilibrium constants for the replacement of *n*-octylamine in $[PdCl_2(C_8H_{17}\cdot NH_2)_2]$ by tri-*n*-butylphosphine have been obtained at 25° in 2 : 2 : 4-trimethylpentane as solvent. This system is characteristic of those useful for studying the equilibria of complexes of metals which do not form simple hydrated ions or of ligands or complexes which are not water-soluble. The absorption spectra of the solutions are used to obtain the concentrations of all three metal-containing compounds in the equilibrium solutions. Since two of the compounds absorb light only in wavelength regions where all three compounds have appreciable extinction coefficients, two new graphical methods for obtaining concentrations from optical-density measurements on mixtures have been developed. The accuracy of each method is shown by application to one set of data, and the results are compared with those obtained by the standard method of solving simultaneous equations.

EQUILIBRIA between complexes of metals which do not form simple hydrated metal ions or of ligands which are insoluble in water or give insoluble complexes, can seldom be studied by conventional methods (see, however, Leden and Chatt [1]). An unreactive organic solvent can be used if a reference ligand other than water is chosen, but it is then necessary to avoid the difficulties caused by ionic association in such liquids. This is most easily done by studying uncharged complexes. The choice of a suitable reference ligand is controlled by several factors : (*a*) The ligand should be monodentate in all circumstances. (*b*) It should co-ordinate to as many different metals as possible. (*c*) To facilitate comparisons between different metals the ligand should form bonds to the metal which are similar for all metals, *i.e.*, "double bonding" and conjugation should be avoided. (*d*) The complexes should be stable and soluble in a wide variety of solvents. (*e*) The free ligand should be readily obtainable, easily purified, and stable.

Nitrogen was considered to be a suitable co-ordinating atom, and a primary aliphatic amine the best class of compound. Availability and consideration of solubility led to the choice of *n*-octylamine. The extensive data existing for the complexes of ammonia in aqueous solution will permit correlation of results in water and other solvents since *n*-octylamine behaves qualitatively in the same way as ammonia, and quantitative studies are possible.

[1] Leden and Chatt, *J.*, 1955, 2936.

Investigations into the nature of the metal–ligand bond have led to increased interest in the complexes formed by atoms of low electronegativity (P, As, Sb, S, Se, Te, etc.), particularly with Group VIII metals. Such complexes can be studied quantitatively in aqueous solution only in special cases. In this paper, a method is described for studying a system which will permit comparison of the strengths of the bonds from the metal to such atoms. Palladium was selected as a suitable metal since it does not give geometrical isomers under the conditions employed, and tri-*n*-butylphosphine as the phosphorus-containing ligand. The acid radical present was chloride and the solvent found to be most suitable was *iso*octane (2 : 2 : 4-trimethylpentane). The system studied can be represented as follows :

$$[PdCl_2(C_8H_{17}\cdot NH_2)_2] + PBu_3 \rightleftharpoons [PdCl_2(C_8H_{17}\cdot NH_2)(PBu_3)] + C_8H_{17}\cdot NH_2$$
$$[PdCl_2(C_8H_{17}\cdot NH_2)(PBu_3)] + PBu_3 \rightleftharpoons [PdCl_2(PBu_3)_2] + C_8H_{17}\cdot NH_2$$

The equilibrium constants $K_1$ and $K_2$, defined as usual, can be used to give the free-energy change on replacement of *n*-octylamine by tri-*n*-butylphosphine. It has been shown [2] that the di-(*n*-octylamine)- and di(tri-*n*-butylphosphine)-palladous chloride complexes are stable in hydrocarbon solvents and that ionisation equilibria are unimportant. In benzene solution the electrical conductance of either of these compounds is unaffected by the presence of excess of ligand, showing that ionic complexes are not formed. The same is true for (*n*-octylamine)(tri-*n*-butylphosphine)dichloropalladium(II) which has also been prepared. Dissociation and chloride-replacement are not important factors influencing the measured constants therefore, but Chatt [3] has pointed out that complexes of Pt(II) containing both an amine and phosphine give evidence of association in carbon tetrachloride solution.[4]

Dr. L. A. Duncanson kindly studied the infrared absorption spectrum of (*n*-octylamine)(tri-*n*-butylphosphine)dichloropalladium(II) in *iso*octane and found two bands due to non-bonded amino-groups at 3355 and 3318 cm.$^{-1}$ and two broad bands due to hydrogen-bonded amino-groups at 3233 and 3149 cm.$^{-1}$. Because of the absorption due to the solvent it was not possible to determine the association constant, but it is of the order of 10. If the total concentration of the complex is $10^{-5}$M, as in the solutions studied here, only about 0·02% will be in the dimeric form, but it is apparent that at higher concentrations any effects due to association will be more significant.

*Method.*—It is necessary to measure the concentrations of as many as possible of the compounds in the equilibrium mixture and a spectrophotometric method appears to be satisfactory. This permits great freedom in the choice of solvent and is of wide applicability since most metal-complex compounds absorb appreciably in the visible or ultraviolet region of the spectrum. Moreover, the optical density of a mixture of compounds is an additive property. Two methods may be used to obtain equilibrium constants from spectrophotometric data : (*a*) if the extinction coefficients of all the compounds present are known then the mixture can be analysed from its absorption spectrum ; (*b*) if the extinction coefficients of only some of the compounds are known then the change of spectrum on change of concentration of one of the ligands can, under suitable conditions, be used to obtain a formation curve (see also method 4 below).

In the system under consideration all the compounds can be isolated, and their extinction coefficients in *iso*octane measured. The Beer–Lambert law was shown to hold for all the metal complexes present in the equilibrium mixture and for mixtures of them. The optical density $D$ at a particular wavelength of a solution containing the five constituents of the equilibrium mixture is, therefore, given by

$$D = l(\varepsilon_1 c_1 + \varepsilon_2 c_2 + \varepsilon_3 c_3 + \varepsilon_n c_n + \varepsilon_p c_p)$$

where $l$ is the length of the light path in the solution and $c_1$, $c_2$, $c_3$, $c_n$, and $c_p$ are respectively the concentrations (moles/l.) of $[PdCl_2(PBu_3)_2]$, $[PdCl_2(PBu_3)(C_8H_{17}\cdot NH_2)]$, $[PdCl_2(C_8H_{17}\cdot NH_2)_2]$, $C_8H_{17}\cdot NH_2$, and $PBu_3$; $\varepsilon_1$, $\varepsilon_2$, $\varepsilon_3$, $\varepsilon_n$, and $\varepsilon_p$ are the molar extinction

[2] Burkin, *J.*, 1954, 71.
[3] Chatt, personal communication.
[4] Chatt, Duncanson, and Venanzi, *Atti R. Accad. Lincei*, 1954, **17**, 120.

coefficients of the respective compounds at that wavelength. In addition, let $M$ moles/l. be the initial concentration of palladium complex used. Since $\varepsilon_p$ is small at all wavelengths used, and it was found that $c_p$ was also small, this equation can be simplified to

$$D = l(\varepsilon_1 c_1 + \varepsilon_2 c_2 + \varepsilon_3 c_3 + \varepsilon_n c_n) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

The extinction coefficients of the compounds at different wavelengths are shown in Fig. 1; $\varepsilon_n$ is too small to be shown on this scale. The data are given in Table 1.

*Calculation of Concentrations.*—The standard, and apparently the simplest, method of obtaining the concentrations of the compounds from the optical densities of the equilibrium solutions is given as method 1. Since the results were not considered to be sufficiently accurate, other graphical methods were used to smooth-out the experimental errors.

*Method* 1. The four extinction coefficients are measured at four suitable wavelengths, and the optical densities of the equilibrium solution at those wavelengths are measured, giving four simultaneous equations from which the four concentrations can be calculated.

*Method* 2. Between 3600 and 2900 Å, $\varepsilon_2$, $\varepsilon_3$, and $\varepsilon_n$ are almost constant, while $\varepsilon_1$ is larger and changes rapidly with wavelength. Then equation (1) can be written

$$D = l(\varepsilon_1 c_1 + f)$$

where $f = \varepsilon_2 c_2 + \varepsilon_3 c_3 + \varepsilon_n c_n$ and is assumed to be constant between these wavelengths. Hence, a plot of $D$ against $\varepsilon_1$ at a series of wavelengths will give a straight line of slope $lc_1$. For wavelengths shorter than 3000 Å the points diverge from the line, and determination of $c_2$ and $c_3$ depends on a quantitative study of the nonlinearity of this portion of the plot. It is obvious, therefore, that since the component of $D$ due to $\varepsilon_1 c_1$ is considerable, even at these shorter wavelengths, the determination of $c_2$ and $c_3$ is very sensitive to experimental errors.

The value of $c_1$ obtained can be used to permit the calculation of $c_2$ and $c_3$ by a similar method. Since the amine is present in large excess, $c_n$ may be taken as the concentration of free amine added in making up the solution, so that equation (1) may be rewritten as

$$D - l\varepsilon_1 c_1 - l\varepsilon_n c_n = l(\varepsilon_2 c_2 + \varepsilon_3 c_3) = \phi$$

where $\phi$ is known. $\varepsilon_2$ is largest compared with $\varepsilon_1$ between 2500 and 3000 Å, and $\phi$ is calculated for a number of wavelengths in this range. From the above equation

$$\phi/\varepsilon_3 = l(\varepsilon_2 c_2/\varepsilon_3 + c_3)$$

and a plot of $\phi/\varepsilon_3$ against $\varepsilon_2/\varepsilon_3$ will give a straight line of slope $lc_2$ and intercept $lc_3$. Similarly a plot of $\phi/\varepsilon_2$ against $\varepsilon_3/\varepsilon_2$ gives a line of slope $lc_3$ and intercept $lc_2$.

*Method* 3. In the previous method, ratios of the extinction coefficients are used to obtain concentrations. Under some conditions it may be preferable to use differences as follows. If $c_1$ has been determined by method 2 and $M$ is known, then

$$M - c_1 = c_2 + c_3 = x$$

and $$c_3 = x - c_2$$

Substitution for $c_3$ in $$\phi = l(\varepsilon_2 c_2 + \varepsilon_3 c_3)$$

gives $$\phi = l(\varepsilon_2 c_2 + \varepsilon_3\{x - c_2\})$$

so that $$\phi = l(c_2\{\varepsilon_2 - \varepsilon_3\} + \varepsilon_3 x)$$

or, since $\varepsilon_3 x$ is known at a particular wavelength,

$$\phi - l\varepsilon_3 x = \alpha = lc_2(\varepsilon_2 - \varepsilon_3)$$

Hence a plot of $\alpha$ against $(\varepsilon_2 - \varepsilon_3)$ over a suitable wavelength region (2500—3000 Å in this case) will give a straight line of slope $lc_2$ passing through the origin.

TABLE 1.

| $\lambda$ (Å) | $D$ * | $\varepsilon_1$ | $\varepsilon_2$ | $\varepsilon_3$ | $\varepsilon_4$ | $\varepsilon_1 c_1$ | $10^3 \varepsilon_4 c_4$ | $\phi$ | $10^4 \phi/\varepsilon_3$ | $\varepsilon_2/\varepsilon_3$ | $10^3 \phi/\varepsilon_2$ | $10^2 \varepsilon_3/\varepsilon_2$ | $\alpha$ | $10^2 \beta$ | $(\varepsilon_2 - \varepsilon_3)$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3600 | 0·304 | 1663 | 710 | 112·3 | — | — | — | — | — | — | — | — | — | — | — |
| 3550 | 0·350 | 1963 | — | 109·0 | — | — | — | — | — | — | — | — | — | — | — |
| 3500 | 0·437 | 2436 | 747 | 110·1 | — | — | — | — | — | — | — | — | — | — | — |
| 3450 | 0·596 | 3355 | 727 | 113·0 | — | — | — | — | — | — | — | — | — | — | — |
| 3400 | 0·875 | 4944 | 757 | 116·9 | 0·0072 | — | — | — | — | — | — | — | — | — | — |
| 3350 | 1·298 | 7460 | 752 | 118·1 | — | — | — | — | — | — | — | — | — | — | — |
| 3300 | 1·864 | 10,840 | 776 | 116·1 | — | — | — | — | — | — | — | — | — | — | — |
| 3280 | 2·140 | 12,160 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 3250 | 2·504 | 14,380 | 792 | 107·5 | — | — | — | — | — | — | — | — | — | — | — |
| 3230 | 2·718 | 15,720 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 3200 | 2·960 | 17,070 | 820 | 97·5 | 0·0126 | — | — | — | — | — | — | — | — | — | — |
| 3170 | 3·048 | 17,650 | 843 | 88·7 | 0·0216 | — | — | — | — | — | — | — | — | — | — |
| 3150 | 3·006 | 17,430 | 865 | 82·1 | — | — | — | — | — | — | — | — | — | — | — |
| 3130 | 2·890 | 16,710 | 872 | — | — | — | — | — | — | — | — | — | — | — | — |
| 3100 | 2·590 | 14,990 | 901 | 69·7 | — | — | — | — | — | — | — | — | — | — | — |
| 3050 | 1·881 | 10,910 | 943 | 58·8 | — | — | — | — | — | — | — | — | — | — | — |
| 3000 | 1·183 | 6711 | 996 | 58·2 | 0·0232 | 1·150 | 1 | 0·032 | 5·502 | 17·12 | 3·21 | 5·84 | 0·030 | −2 | 938 |
| 2950 | 0·704 | 3827 | 1143 | 68·6 | — | 0·656 | 1 | 0·047 | 6·851 | 16·66 | 4·11 | 6·00 | 0·045 | −13 | 1074 |
| 2900 | 0·467 | 2423 | 1500 | 101·4 | 0·0443 | 0·415 | 2 | 0·050 | 4·931 | 14·79 | 3·33 | 6·76 | 0·047 | −6 | 1399 |
| 2850 | 0·400 | 1854 | 2349 | 166·6 | 0·0697 | 0·318 | 3 | 0·079 | 4·742 | 14·10 | 3·36 | 7·09 | 0·074 | −9 | 2182 |
| 2800 | 0·404 | 1642 | 3832 | 288·1 | 0·131 | 0·281 | 6 | 0·117 | 4·061 | 13·30 | 3·05 | 7·52 | 0·109 | −2 | 3544 |
| 2770 | 0·408 | 1544 | 4929 | 401·1 | — | 0·265 | 7 | 0·136 | 3·391 | 12·29 | 2·76 | 8·14 | 0·124 | 11 | 4528 |
| 2750 | 0·406 | 1428 | 5637 | 490·7 | 0·181 | 0·245 | 8 | 0·153 | 3·118 | 11·49 | 2·71 | 8·71 | 0·138 | 15 | 5146 |
| 2730 | 0·400 | 1295 | 6296 | 593·2 | — | 0·222 | 8 | 0·170 | 2·866 | 10·61 | 2·70 | 9·42 | 0·152 | 18 | 5703 |
| 2720 | 0·395 | 1221 | 6543 | 664·0 | — | 0·209 | 9 | 0·177 | 2·666 | 9·85 | 2·71 | 10·15 | 0·157 | 18 | 5879 |
| 2710 | 0·389 | 1166 | 6766 | 721·2 | — | 0·200 | 10 | 0·179 | 2·482 | 9·38 | 2·65 | 10·66 | 0·168 | 23 | 6045 |
| 2700 | 0·380 | 1133 | 6918 | 792·6 | 0·229 | 0·194 | 10 | 0·176 | 2·221 | 8·73 | 2·54 | 11·46 | 0·152 | 31 | 6125 |
| 2690 | 0·372 | 1064 | 6987 | 872·4 | — | 0·182 | 10 | 0·180 | 2·063 | 8·01 | 2·58 | 12·49 | 0·154 | 29 | 6115 |
| 2680 | 0·364 | 995 | 7036 | 907·3 | 0·246 | 0·171 | 10 | 0·183 | 2·017 | 7·76 | 2·60 | 12·89 | 0·156 | 27 | 6129 |
| 2670 | 0·354 | 948 | 6987 | 1031 | — | 0·162 | 10 | 0·182 | 1·765 | 6·78 | 2·61 | 14·76 | 0·151 | 27 | 5956 |
| 2660 | 0·344 | 913 | 6918 | 1105 | 0·263 | 0·156 | 10 | 0·178 | 1·611 | 6·26 | 2·57 | 15·97 | 0·145 | 29 | 5813 |
| 2650 | 0·335 | 886 | 6766 | 1201 | — | 0·152 | 11 | 0·172 | 1·432 | 5·63 | 2·54 | 17·75 | 0·136 | 30 | 5565 |
| 2630 | 0·319 | 837 | 6249 | 1381 | 0·287 | 0·143 | 12 | 0·164 | 1·187 | 4·52 | 2·63 | 22·11 | 0·123 | 23 | 4868 |
| 2600 | 0·308 | 891 | 5265 | 1631 | 0·311 | 0·153 | 13 | 0·142 | 0·871 | 3·23 | 2·70 | 30·97 | 0·093 | 15 | 3634 |
| 2580 | 0·316 | 1010 | 4566 | 1788 | 0·336 | 0·173 | 14 | 0·129 | 0·721 | 2·55 | 2·83 | 39·2 | 0·076 | 7 | 2778 |
| 2560 | 0·344 | 1226 | 3897 | 1940 | 0·368 | 0·210 | 16 | 0·118 | 0·605 | 2·01 | 3·03 | 49·8 | 0·060 | −1 | 1957 |
| 2540 | 0·395 | 1581 | 3286 | 2062 | 0·415 | 0·271 | 18 | 0·106 | 0·514 | 1·59 | 3·23 | 62·7 | 0·044 | −8 | 1224 |
| 2520 | 0·474 | 2050 | 2910 | 2181 | 0·494 | 0·351 | 21 | 0·102 | 0·468 | 1·33 | 3·51 | 75·0 | 0·037 | −15 | 729 |
| 2500 | 0·590 | 2684 | 2727 | 2335 | 0·631 | 0·460 | 27 | 0·103 | 0·441 | 1·17 | 3·78 | 85·6 | 0·033 | −22 | 392 |

* Optical densities refer to a 1·0 cm. cell.

Similar substitution for $c_2$ and subsequent rearrangement (since $\epsilon_2 > \epsilon_3$ throughout the wavelength region used) gives

$$l\epsilon_2 x - \phi = \beta = lc_3(\epsilon_2 - \epsilon_3)$$

*Method* 4. This method for obtaining the equilibrium constants may be used when it is possible to measure the concentration of only one of the complexes present in the equilibrium mixture. It requires data for a number of different equilibrium solutions with a range of concentrations of the determinable constituent. For this reason it was not used in the present investigation, the simpler methods being adequate.

Since di-(*n*-octylamine)dichloropalladium(II) has no appreciable absorption peak except at wavelengths shorter than 2500 Å, where all palladium complexes of this type absorb, this compound will not be the one which is determined. It is convenient, therefore, to re-write the equilibria and put $p_2 = 1/K_1$ and $p_1 = 1/K_2$. It is apparent that for a solution containing a given quantity of bis-phosphine complex and free amine mixed together there will exist a relation between these concentrations, the amount of phosphine complex converted into the other two complexes, and the two equilibrium constants. It is assumed that the bis-phosphine complex is the constituent which can be determined, so that $M$ and $c_1$ are known, and hence the amount of the compound converted into the phosphine-amine and bis-amine complexes ($x$). Then

$$p_1 = (x - c_3)(c_p)/c_1c_n$$

and since

$$c_p = c_2 + 2c_3 = x + c_3$$

$$p_1c_1c_n = (x - c_3)(x + c_3)$$

and

$$c_3^2 = x^2 - p_1c_1c_n$$

Similar manipulation of the equation for $p_2$ gives

$$p_2c_nx - c_3(p_2c_n + x) - c_3^2 = 0$$

Substitution for $c_3$ and $c_3^2$ in terms of $p_1$ then gives

$$p_2c_nx - (p_2c_n + x)(x^2 - p_1c_1c_n)^{\frac{1}{2}} - (x^2 - p_1c_1c_n) = 0 \quad . \quad . \quad . \quad (2)$$

The following treatment of this equation was suggested by Mr. E. W. Balson. An upper limit is set for $p_1$ by $p_1 \geqslant x^2/c_1c_n$. Putting $x^2 - p_1c_1c_n = \psi$, we can write (2) as

$$p_2c_nx - (p_2c_n + x)\psi^{\frac{1}{2}} - \psi = 0$$

$$\therefore \quad p_2(c_nx - c_n\psi^{\frac{1}{2}}) - x\psi^{\frac{1}{2}} - \psi = 0$$

and

$$p_2 = (x\psi^{\frac{1}{2}} + \psi)/c_n(x - \psi^{\frac{1}{2}})$$

From a series of equilibrium solutions a set of limiting values for $p_1$ can be found. Setting $p_1$ equal to a series of arbitrary values less than these limiting values, we can calculate a set of values for $p_2$. A value of $p_1$ is found for which the spread in the values of $p_2$ is least. Alternatively, for a given equilibrium solution several possible values of $p_1$ can be chosen, the corresponding values of $p_2$ calculated, and the results plotted with $p_1$ and $p_2$ as ordinates. If the curves for a number of solutions are plotted together, the point of intersection will give the true values of $p_1$ and $p_2$.

## Discussion and Results

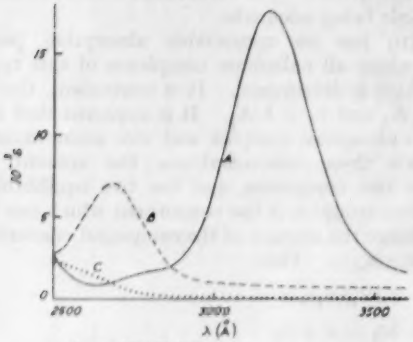
Detailed results are presented (Table 1) for one solution in which the concentrations of the compounds were such that convenient optical densities were obtained over the whole wavelength range by using 5, 10, and 20 mm. cells. In many other cases the solution had to be diluted in order to obtain $c_1$. In order to show the value and limitations of the different methods described above, the data are treated by each method separately.

*Method* 1. By using the data for 3170, 2680, and 2500 Å, and correcting $D$ for $\epsilon_nc_n$, those combinations of the equations considered to be most likely to be reliable gave the results shown in Table 3. The minimum total concentration is $2{\cdot}115 \times 10^{-4}$ which is 105% of $M$.

*Method* 2. The excellent straight line obtained by plotting $\varepsilon_1$ against $D$ between 3600 and 3000 Å gives $c_1 = 1{\cdot}713 \times 10^{-4}$. It was found that small amounts of impurities or other systematic errors in the determination of $\varepsilon_1$ caused non-linearity or, frequently, two parallel lines, one for wavelengths below 3170 Å and the other for longer wavelengths.

Figs. 2 and 3 show the results plotted to obtain $c_2$ and $c_3$. In a consideration of these

FIG. 1.




*A*, $\varepsilon_1$, $[PdCl_2(PBu_3)_2]$
*B*, $\varepsilon_2$, $[PdCl_2(PBu_3)(C_6H_{17}{\cdot}NH_2)]$
*C*, $\varepsilon_3$, $[PdCl_2(C_6H_{17}{\cdot}NH_2)_2]$

FIG. 2.

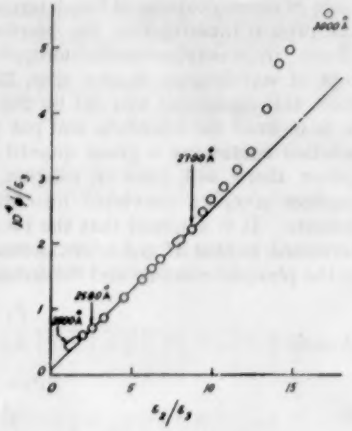
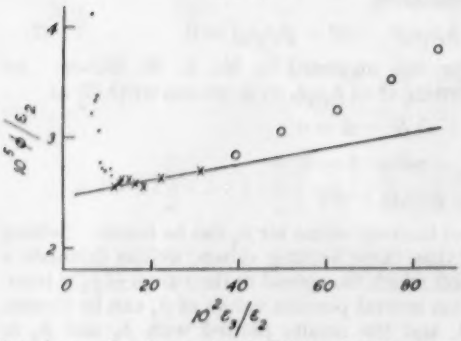
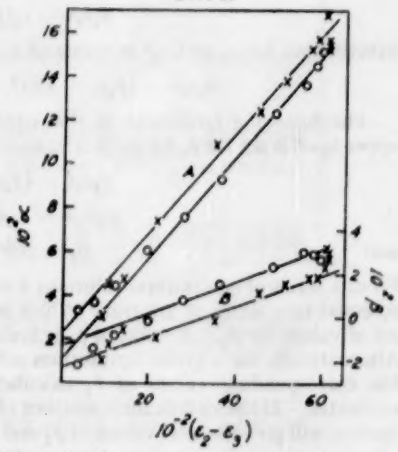



FIG. 3.




*Data for wavelengths* : ●, 3000—2710; ×, 2700—2600; ○, 2580—2500 Å.

FIG. 4.




*Lines A refer to α, B to β. Data for wavelengths* : ×, 3000—2680; ○, 2670—2500 Å.

and Fig. 4, the following facts are significant : (*a*) at wavelengths longer than 2850 Å, $\varepsilon_1$ is rising rapidly while $\varepsilon_2$ and $\varepsilon_3$ are falling slightly or are constant; (*b*) between 2850 and 2700 Å $\varepsilon_1$ changes little; (*c*) $\varepsilon_2$ is a maximum at about 2680 Å.

Fig. 2 shows a good straight line between 2700 and 2580 Å, the slope of which gives $c_2 = 2{\cdot}47 \times 10^{-5}$, and the intercept, $c_3 = 0{\cdot}7 \times 10^{-5}$. Putting the intercept equal to $2{\cdot}47 \times 10^{-5}$ in Fig. 3 we can draw the best line for the results between 2700 and 2580 Å, the slope giving $c_3 = 0{\cdot}70 \times 10^{-5}$. The large deviation from the line of the results at wavelengths outside this region is due to normal experimental error magnified by the method of

plotting. This is shown by considering separately the three possible sources of error. (*a*) $\phi$ in error. The value of $\phi$ which would give linearity over the whole of the wavelength region between 2500 and 3000 Å can be calculated from Figs. 2 and 3. The error ($\Delta\phi$) in $\phi$ is the same in each case and can be due either to an error in the optical density equal to $\Delta\phi$, or to an error in either $\varepsilon_1 c_1$ or $\varepsilon_n c_n$, best expressed as a percentage error. In Table 2 $\Delta\phi$ has been expressed as a percentage of $\varepsilon_1 c_1$. (*b*) $\varepsilon_2$ in error. From Fig. 2 the value of $\varepsilon_2/\varepsilon_3$ and hence of $\varepsilon_2$ necessary for linearity can be calculated. (*c*) $\varepsilon_3$ in error. The value of $\varepsilon_3$ necessary for linearity can be obtained similarly from Fig. 3.

Examination of Fig. 3 and the calculated corrections expressed as $100\varepsilon_{obs.}/\varepsilon_{calc.}$ (Table 2) shows that the source of error is most unlikely to be the value of $\varepsilon_3$. Although an error of less than 10% in $\varepsilon_2$ would give linearity between 2770 and 2710 Å, much greater errors are necessary to correct the data for longer wavelengths. The most probable source of error appears, therefore, to be $\phi$. Of the terms comprising this, $\varepsilon_n$ is so small that it cannot be the source of error (see Table 2). Although the optical-density measurements were generally reproducible to 0·005, the possible experimental error was considered to be $\pm$0·01 and is likely to be greatest where $D$ is changing rapidly with wavelength since both prism-setting and slit-width are then important factors. This error will not wholly account for $\Delta\phi$, but since a 1% error in either $\varepsilon_1$ or $c_1$ would eliminate the residue, it is considered that the deviations from linearity shown in Figs. 2 and 3 are due to normal experimental errors in determining the optical density of the equilibrium solution or the solutions used to obtain $\varepsilon_1$.

TABLE 2.

| λ (Å) | $\Delta\phi$ | $D$ | $10^3\varepsilon_n c_n$ | $\varepsilon_1 c_1$ | $\frac{100\Delta\phi}{\varepsilon_1 c_1}$ | $\frac{100\varepsilon_2(\text{obs.})}{\varepsilon_2(\text{calc.})}$ | $\frac{100\varepsilon_3(\text{obs.})}{\varepsilon_3(\text{calc.})}$ |
|---|---|---|---|---|---|---|---|
| 3000 | 0·007 | 1·183 | 1 | 1·150 | 0·6 | 77·8 | 7·1 |
| 2900 | 0·012 | 0·467 | 2 | 0·412 | 2·9 | 75·1 | 6·8 |
| 2800 | 0·020 | 0·404 | 6 | 0·281 | 7·1 | 82·2 | 12·0 |
| 2750 | 0·010 | 0·406 | 8 | 0·245 | 4·1 | 93·8 | 34·3 |
| 2730 | 0·010 | 0·400 | 8 | 0·222 | 4·5 | 94·3 | 38·9 |
| 2720 | 0·009 | 0·395 | 9 | 0·209 | 4·3 | 93·8 | 41·3 |
| 2710 | 0·006 | 0·389 | 10 | 0·200 | 3·0 | 96·2 | 58·6 |

*Method* 3. The value of $\varepsilon_1$ was taken as $1{\cdot}713 \times 10^4$, giving $x = 2{\cdot}99 \times 10^{-5}$. Plotting $\alpha$ or $\beta$ against $(\varepsilon_2 - \varepsilon_3)$ gives two parallel lines in each case, the points for wavelengths between 2720 and 2650 Å scattering between the two lines. This region corresponds to the peak of $\varepsilon_2$ and suggests that there is a systematic error in $\varepsilon_2$ on one side of the absorption peak. Such an error could be due, *e.g.*, to slight disproportionation of the phosphine–amine complex, used to determine $\varepsilon_2$. If the value of $\alpha$ at 2850 Å is to lie on the line drawn for wavelengths below 2600 Å (Fig. 4), then $\varepsilon_2$ must be approximately 2800, not 2349, giving $(\varepsilon_2 - \varepsilon_3) = 2633$. The amended value of $\beta$ is then 0·0047 and the data for 2850 Å fall precisely on the line drawn for wavelengths below 2650 Å. It is apparent, therefore, that the existence of two parallel lines in this method can be explained quantitatively in terms of a systematic error in $\varepsilon_2$ on one side of the absorption peak. Since the lines are parallel, however, the values of $c_2$ and $c_3$ obtained are not affected. The lines do not pass through the origin, and this appears to be due largely to errors in $\alpha$ and $\beta$, but, again the slopes are not affected. Deviations from the lines occur at wavelengths above 2850 and below 2560 Å for reasons similar to those discussed under method 2. The value of $c_2$ obtained from Fig. 4 is $2{\cdot}29 \times 10^{-5}$, and Fig. 6 gives $c_3 = 0{\cdot}75 \times 10^{-5}$.

*Summary of Results.*—The results obtained for the equilibrium solution discussed above are given in Table 3. It is apparent that the value of $c_3$ obtained by method 1 is valueless, and $c_2$ depends on the wavelength chosen for the calculations, being fairly reliable. The values of $c_2$ obtained by methods 2 and 3 differ considerably, and although that from the former is probably the more reliable, the total concentration from the latter is closer to $M$. Values of $K_1$ and $K_2$ are, therefore, calculated from both sets of figures.

Other equilibrium solutions gave results essentially similar to those detailed above.[5] Three separate solutions were studied by the techniques described below, each being diluted sufficiently to give suitable optical densities within the wavelength ranges used to measure

[5] Meddings, Thesis, Southampton Univ., 1955.

TABLE 3.

| Method | $10^5c_1$ | $10^5c_2$ | $10^5c_3$ | $10^5c_p$ | $100(c_1 + c_2 + c_3)/M$ | $10^{-3}K_1$ | $10^{-3}K_2$ |
|---|---|---|---|---|---|---|---|
| 1 | 1·713 | 2·46 | 2·02 | — | — | — | — |
| | | 2·42 | 1·60 | | 105 | | |
| | | | 1·85 | | | | |
| 2 | 1·713 | 2·47 | 0·7 | — | — | — | — |
| | | | 0·70 | 3·87 | 101 | 3·93 | 7·72 |
| 3 | — | 2·29 | 0·75 | 3·79 | 100 | 3·47 | 5·50 |

$M = 2{\cdot}012 \times 10^{-4}$; $c_a = 4{\cdot}306 \times 10^{-2}$.

the three concentrations. Pairs of solutions from the same stock solution are bracketed together, and in each case $c_1$ was obtained from the more dilute solution. Concentrations used in calculating the equilibrium constants were obtained by method 2, since $c_2$ and $c_3$ obtained by method 3 were similar but subject to some uncertainty in choice of the line through the experimental points.

| | | | | | |
|---|---|---|---|---|---|
| $10^4M$ | 2·012 | 6·946 | 2·510 | 2·484 | 1·374 |
| $10^5c_2$ | 4·306 | 21·01 | 7·59 | 2·064 | 1·142 |
| $\log K_1$ | 3·59 | 3·42 | 3·34 | 3·45 | 3·72 |
| $\log K_2$ | 3·89 | 4·12 | 4·34 | 3·98 | — |

Hence the equilibrium constants for the system at 25° are

$$\log K_1 = 3{\cdot}34 - 3{\cdot}72; \quad -\Delta G_1 = 4{\cdot}56 - 5{\cdot}07 \text{ kcal.}$$

$$\log K_2 = 3{\cdot}89 - 4{\cdot}34; \quad -\Delta G_2 = 5{\cdot}30 - 5{\cdot}92 \text{ kcal.}$$

## EXPERIMENTAL

The spectrophotometer used was the Hilger "Uvispek." The wavelength drum was checked for accuracy periodically, and the density scale was tested by using pure potassium nitrate solutions of different concentrations in 0·5 and 1·0 cm. cells, at 3010 Å. Plotting the extinction coefficient found for each solution against the optical density should give a straight line parallel to the density axis. It was found that optical density values smaller than 0·4 became increasingly unreliable (usually too high), and that values larger than 1·3 are also unreliable (too low). Accordingly, wherever possible, optical density measurements were confined to the range $D = 0{\cdot}4$—$1{\cdot}3$. The silica absorption cells were cleaned in hot detergent ("Teepol") solutions, thoroughly rinsed in running hot water and a jet of distilled water, and dried *in vacuo* over phosphoric oxide.

It was found that the tributylphosphine in the equilibrium mixture was decomposed by oxygen or water, so the solutions were prepared and studied in an inert atmosphere. A new cell holder for the spectrophotometer was made, therefore, so that the cells could be kept air-tight and maintained at a constant temperature. A block of high-purity copper was used, drilled so that water pumped from a constant-temperature bath circulated through the walls of the holder, keeping the temperature constant within $\pm 0{\cdot}1°$. The bottom of each cell compartment was covered by a strip of soft thin rubber, and the undersurfaces of the cell lids were ground flat and covered with a cellulose adhesive tape. The mild-steel cover of the cell holder screwed down, and pressure screws on to the individual cell lids could be tightened to make gas-tight seals. Solutions were prepared and the cells filled and sealed in a "manipulation box" containing commercial nitrogen and a residual air concentration of less than 0·1% of the gas present.

Preliminary experiments indicated that, if appreciable concentrations of amine-containing complexes were to be present in the equilibrium mixture, the concentration of tributylphosphine present must be very small and the amount of *n*-octylamine relatively large. These requirements were most easily met by using the bis-phosphine–palladium complex and adding amine. In view of the small quantities of the complex required and the relative complexity of the technique used to make up the reaction solutions, stock solutions of the materials were made up for each set of mixtures studied. To increase the accuracy with which the starting concentrations were known, the quantities of stock solutions and solvent used were weighed. If suitable precautions are taken, this is probably unnecessary now that the validity of the methods used to treat the data is established.

Stock solutions of the phosphine complex were prepared in a stoppered volumetric flask by weighing solid and solvent. Stock solutions of *n*-octylamine were prepared as follows. Pure redistilled amine was again distilled at 16 mm. immediately before use, the condenser passing

through a sealed hole into the manipulation box. The requisite volume of amine was added to a weighed quantity of *iso*octane in a weighed Pyrex-glass tube with a constriction a little below the top. A quill tube inside a second tube was used for the introduction to avoid wetting the top or neck of the tube. Into the constriction was put a piece of glass rod which fitted tightly but did not fall through. The tube was suspended by a clamp above the constriction, and a coil of nichrome wire wound around this was connected through a suitable resistance to the A.C. mains. As the glass softened, the weight of the bulb and contents caused the neck to pull out until it was in contact with the rod, which then softened and sealed the bulb. This was later removed from the box and weighed to obtain the weight of amine present.

The tubes in which equilibrium was attained were similar but had a second constriction at the top. After they had been cleaned and dried by repeated evacuation and introduction of dried air, the tubes were evacuated to $10^{-2}$ mm. and tested to ensure that no pinholes were present, and the top constriction was sealed, a deep scratch being made later. Each tube was put in a wire cradle so that it would stand upright and had a piece of rubber tubing sealed with glass rod fitted over the sealed constriction to act as a stopper later. The tube, stopper, and rod to be used for sealing the lower constriction were weighed together and a number of such sets were introduced into the manipulation box. The top seal was broken off at the scratch and a measured amount of the stock phosphine complex was introduced through a quill and guard tube. The tube was stoppered, removed from the box together with the glass fragment broken off, and weighed. It was put back into the box, amine solution was added in the same way, and the tube removed and weighed. In each case the stoppered tube was in the air for only a few minutes. It was then sealed, or the mixture was divided into several portions by pouring into other tubes in the box, each of these being sealed by electric heating as described above, removed from the box, re-sealed in a flame just below the stopper, and kept at 25°. When necessary, the solutions were further diluted by a similar technique before measurements were made. The absorption cells were filled and clamped in the cell holder in the nitrogen-filled box.

Solutions of the bis-phosphine, phosphine-amine, and bis-amine complexes to be used for measurement of the extinction coefficients were prepared by weighing in the open laboratory. To obtain appreciable optical-density values, the concentration of *n*-octylamine used in the determination of $\varepsilon_n$ had to be approximately 100 times the concentration present in an equilibrium mixture.

Since all solutions were prepared by weight while concentrations were required to be expressed in moles/l., it was shown that mixtures of *n*-octylamine and *iso*octane containing up to 38% of amine show a linear relationship between amine concentration and density.

*Materials.*—*iso*Octane (2 : 2 : 4-trimethylpentane) was obtained from the Esso refinery at Fawley, Hants. It was redistilled before use; $d^{20}$ 0·6877.

n-*Octylamine*. The commercial material from Armour and Co. was fractionated *in vacuo* several times, b. p. data given by Ralston *et al.*[6] being accepted.

*Di*-(n-*octylamine*)*dichloropalladium*(II). Palladous chloride (2·7 g.) and ammonium chloride (1·63 g., 2 mol.) were dissolved in water (150 c.c.) in a 250 c.c. stoppered bottle, and *n*-octylamine (4·0 g., 2 mol.) added. After 2 hours' shaking, the precipitated *complex* was filtered off, dried, and recrystallised from light petroleum (b. p. 80—100°); it had m. p. 112·5° (Found: C, 44·5; H, 8·6; N, 6·6. $C_{16}H_{38}N_2Cl_2Pd$ requires C, 44·1; H, 8·8; N, 6·4%).

(*Tri*-n-*butylphosphine*)(n-*octylamine*)*dichloropalladium*(II). Di(tri-*n*-butylphosphine)dichloro-μ-dichlorodipalladium(II) (1·906 g.) was dissolved in the minimum amount of warm benzene, and *n*-octylamine (0·643 g., 2·00 mol.) added to the cooled solution. The benzene was removed *in vacuo*, leaving the required *complex* as a viscous, golden-yellow oil. Attempts to crystallise this by cooling it, or its solutions in many solvents, to −40° were unsuccessful, but disproportionation occurred in some solvents, the phosphine complex crystallising (Found: C, 46·6; H, 9·1; N, 3·0. $C_{20}H_{46}NCl_2PPd$ requires C, 47·2; H, 9·1; N, 2·75%). The absorption spectrum proves that the compound is not a mixture of the bis-phosphine and bis-amine complexes and contains no bridged compound.

This work was carried out in the Department of Chemistry, Southampton University, during the tenure of a University research award by one of us (B. M.).

Imperial College of Science, London, S.W.7.
[Present address (B. M.): Organic Chemistry Research Dept., Imperial Chemical Industries Limited, Billingham.] [*Received, August 16th*, 1955.]

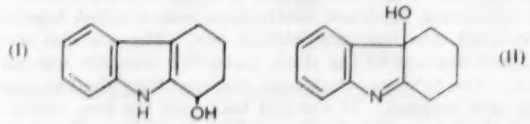
[6] Ralston, Selby, Pool, and Potts, *Ind. Eng. Chem.*, 1940, **32**, 1093.

## 233. *Attempted Synthesis of* 1 : 9-9′ : 1′-*Dicarbazolylene.*

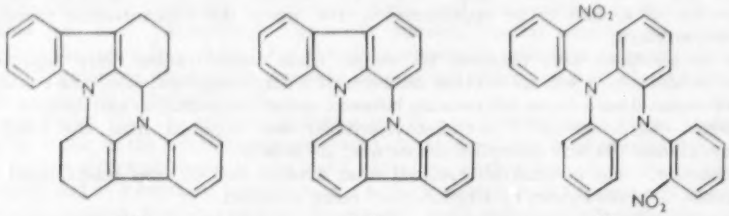
By Ann C. Geale, June M. G. Linnell, and Muriel L. Tomlinson.

The preparations of a number of derivatives of 1 : 9′-dicarbazolyl are described.

The substance $C_{12}H_{13}ON$, obtained from the product of bromination of certain acyltetrahydrocarbazoles [1] and previously thought to be 2 : 3 : 4 : 11-tetrahydro-11-hydroxycarbazole was shown [2] to be 1 : 2 : 3 : 4-tetrahydro-1-hydroxycarbazole (I), and an isomeric compound obtained from bromination of 1 : 2 : 3 : 4-tetrahydroxycarbazole itself,[3] formerly regarded as 1 : 2 : 3 : 10-tetrahydro-10-hydroxycarbazole, was found to be tetrahydro-11-hydroxycarbazolenine (II).[2] Both the compounds (I) and (II), on dehydration, gave a




substance originally thought to be 2 : 3-dihydrocarbazole, but as dehydrogenation of the so-called " dihydrocarbazole " afforded a substance $C_{24}H_{14}N_2$, and not carbazole, it became clear that the compound $C_{24}H_{14}N_2$ is 1 : 9-9′ : 1′-dicarbazolylene (IV) and that it had been formed from 1 : 2 : 3 : 4 : 1′ : 2′ : 3′ : 4′-octahydro-1 : 9-9′ : 1′-dicarbazolylene (III).[3] It would be desirable to confirm these structures by synthesis of the compounds (III) or (IV) and a number of experiments have been carried out to explore the possibilities of synthesising the latter.



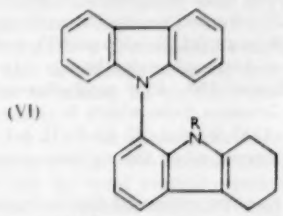

Wieland and Lecher [4] found that when 4-nitro-*N*-nitrosodiphenylamine was decomposed by boiling it in xylene, 9 : 10-dihydro-9 : 10-di-*p*-nitrophenylphenazine was among the products. It was hoped that, in a similar fashion, 2-nitro-*N*-nitrosodiphenylamine might afford 9 : 10-dihydro-9 : 10-di-*o*-nitrophenylphenazine (V), which could then, perhaps, by reduction and diazotisation, be converted into (IV).

In fact 2-nitro-*N*-nitrosodiphenylamine gave a complex mixture (shown by adsorption on alumina) : the main product was 2 : 4′-dinitrodiphenylamine and the only other crystalline material isolated was some 2-nitrodiphenylamine. (It was later found that Juillard [5] had recorded the production of 2 : 4′-dinitrodiphenylamine by heating this nitrosamine in light petroleum.)

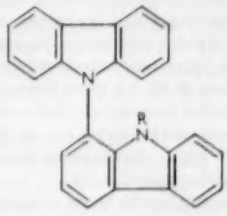
Attention was then turned to the possibility of dehydrogenating 1-9′-carbazolyl-5 : 6 : 7 : 8-tetrahydrocarbazole (VI; R = H), 1 : 9′-dicarbazolyl (VII; R = H), or more highly reduced derivatives of this type. Condensation of 9-*o*-aminophenylcarbazole with

[1] Plant and Tomlinson, *J.*, 1931, 3324.
[2] *Idem*, *J.*, 1950, 2127.
[3] *Idem*, *J.*, 1933, 298.
[4] Wieland and Lecher, *Annalen*, 1912, **392**, 165.
[5] Juillard, *Bull. Soc. chim.* (*France*), 1905, **33**, 1181.

2-hydroxy*cyclo*hexanone proceeded only as far as 9-*o*-(2-oxo*cyclo*hexylamino)phenylcarbazole which could not be cyclised to give the compound (VI; R = H), but this was prepared by the Fischer process from 9-*o*-hydrazinophenylcarbazole and *cyclo*hexanone. It was dehydrogenated smoothly to the desired compound (VII; R = H). Further dehydrogenation, to the dicarbazolylene (IV), could not be brought about by any of the standard methods, such as heating with active palladium catalysts or with sodium aluminium chloride.

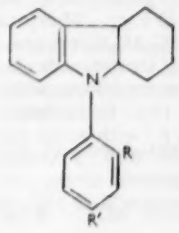


(VI)

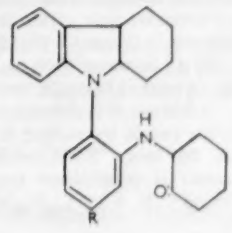


(VII)

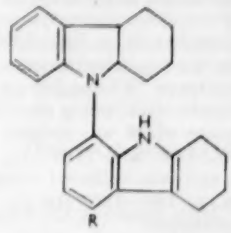
It was, nevertheless, still thought worth while to explore the possibility of cyclising compounds such as (X) during dehydrogenation. It was not possible to condense tetrahydrocarbazole with any *o*-halogenonitrobenzene that was investigated, nor did 1 : 2 : 3 : 4 : 10 : 11-hexahydrocarbazole react with *o*-chloronitrobenzene, but hexahydrocarbazole condensed smoothly with the more reactive 1-chloro-4-cyano-2-nitrobenzene to give 9-(4-cyano-2-nitrophenyl)-1 : 2 : 3 : 4 : 10 : 11-hexahydrocarbazole (VIII; R = $NO_2$, R′ = CN). The intention was then to convert this into a 9-(1 : 2 : 3 : 4-tetrahydro-8-carbazolyl)-1 : 2 : 3 : 4 : 10 : 11-hexahydrocarbazole (X; R = H or $CO_2H$ or CN) which might ultimately be made into the dicarbazolylene (IV). The compound (VIII; R = $NO_2$,



(VIII)



(IX)



(X)

R′ = CN) was reduced to the amine (VIII; R = $NH_2$, R′ = CN) and a small quantity of 9-(5-cyano-1 : 2 : 3 : 4-tetrahydro-8-carbazolyl)-1 : 2 : 3 : 4 : 10 : 11-hexahydrocarbazole (X; R = CN) was obtained from this amine and 2-hydroxy*cyclo*hexanone, but the reaction appeared to be capricious and unlikely to yield sufficient material to allow the final cyclisation to be investigated. No greater success was achieved with the acid (VIII; R = $NH_2$, R′ = $CO_2H$) obtained by the hydrolysis of the aminonitrile: it could not be converted into a hydrazino-acid, and condensation with 2-hydroxy*cyclo*hexanone proceeded only as far as the formation of 9-(4-carboxy-2-2′-oxo*cyclo*hexylaminophenyl)-1 : 2 : 3 : 4 : 10 : 11-hexahydrocarbazole (IX; R = H) and so this route was abandoned.

1 : 9′-Dicarbazolyl was converted into 9-nitroso-1 : 9′-dicarbazolyl (VII; R = NO) but this did not give the dicarbazolylene (IV) on pyrolysis. Plant and Duthie [6] found that pyrolysis of polycyclic methyl indole-*N*-carboxylates may result in the loss of carbon dioxide to give mixtures of the *N*-methylindole and the indole itself. Various methods were therefore explored in the hope of converting the compounds (VI and VII; R = H)

[6] Plant and Duthie, *J.*, 1952, 1899.

into the esters (VI and VII; R = $CO_2Me$) because pyrolysis of these substances might produce the dicarbazolylene (IV), but the hindered NH group failed to react as desired.

## Experimental

9-o-*Nitrophenylcarbazole.*—Dunlop and Tucker's method [7] was modified by mixing the carbazole (20 g.), *o*-chloronitrobenzene (40 g.), dry potassium carbonate (20 g.), and copper bronze (0·3 g.) with nitrobenzene (25 c.c.) and refluxing for 5 hr. (yield, 60—65%).

9-o-*Aminophenylcarbazole.*—Reduction of the above nitro-compound, in fine suspension in alcohol, with Adams catalyst and hydrogen at N.T.P. afforded nearly theoretical yields of the pure amine. This method is convenient in small scale work (cf. Dunlop and Tucker [7]).

9-(o-2′-*Oxocyclohexylaminophenyl)carbazole.*—9-o-Aminophenylcarbazole (1·3 g.) and 2-hydroxy*cyclo*hexanone (0·55 g.) were heated together at 110—130° until effervescence ceased (20—30 min.). The solid formed crystallised from benzene from which 9-(o-2′-*oxocyclohexylaminophenyl)carbazole* separated as plates, m. p. 156—158° (Found: C, 81·6; H, 6·1. $C_{24}H_{22}ON_2$ requires C, 81·3; H, 6·2%). No further reaction occurred when this substance was heated to 300° with a trace of hydrochloric acid.

9-o-*Hydrazinophenylcarbazole Hydrochloride.*—A fine suspension of the hydrochloride made by warming 9-*o*-aminophenylcarbazole (30 g.) with hydrochloric acid (150 c.c.) and water (30 c.c.) was diazotised at −10° to −5° with sodium nitrite (10 g.) in water (50 c.c.). The deep red diazonium solution was kept at this temperature for ½ hr. and then added, with good stirring, to stannous chloride (65 g.) in hydrochloric acid (100 c.c.) at 0°. The crude hydrazine salt was collected after 4 hr., the colour having changed from red to pale brown. To purify the salt, it was suspended in boiling dilute hydrochloric acid, and hydrogen sulphide was passed in to precipitate the tin. 9-o-*Hydrazinophenylcarbazole hydrochloride* was obtained from the filtrate as plates, m. p. 222—224° (decomp.) (Found: C, 70·0; H, 5·5. $C_{18}H_{15}N_3$,HCl requires C, 69·8% H, 5·2%).

1-9′-*Carbazolyl*-5 : 6 : 7 : 8-*tetrahydrocarbazole.*—The above pure hydrazine hydrochloride (3 g.), suspended in alcohol (25 c.c.), was treated with ammonia (1·5 c.c.; *d* 0·88) which precipitated the hydrazine as a solid. To this was added *cyclo*hexanone (1 g.) and, after warming and stirring, 9-o-cyclo*hexylidenehydrazinophenylcarbazole* crystallised (3·1 g.). It crystallised from alcohol as needles, m. p. 109—120° (decomp.) (Found: N, 12·2. $C_{24}H_{23}N_3$ requires N, 11·9%). Alternately the crude hydrazine salt (25 g.) in water (500 c.c.) was treated with ammonia until alkaline to litmus. The hydrazine was extracted from the resulting precipitate (which contained tin compounds) with boiling alcohol. Addition of *cyclo*hexanone (10 g.) to this solution afforded the hydrazone which was cyclised to the indole by boiling it (24 g.) with acetic acid (240 c.c.) and hydrochloric acid (110 c.c.) for 20 min. 1-9′-*Carbazolyl*-5 : 6 : 7 : 8-*tetrahydrocarbazole* separated and was collected when cold; it crystallised from aqueous acetic acid (75%) as prisms, m. p. 194—196° (18 g.) (Found: N, 8·3. $C_{24}H_{20}N_2$ requires N, 8·3%). No picrate could be obtained.

1 : 9′-*Dicarbazolyl.*—The above tetrahydro-compound (10 g.) was heated with 10% palladised charcoal at 235—240° in a stream of carbon dioxide for 2 hr. Extraction with boiling alcohol and precipitation with water afforded 1 : 9′-*dicarbazolyl* (7·5 g.). It crystallised from alcohol as plates, m. p. 191°, depressed by admixture with the preceding substance (Found: C, 86·5; H, 4·8; N, 8·6. $C_{24}H_{16}N_2$ requires C, 86·7; H, 4·8; N, 8·4%). Mixed in hot alcoholic solution with picric acid it gave a dark red *picrate* which crystallised from alcohol as prisms, m. p. 140—142° (decomp.) (Found: C, 55·1; H, 2·8. $C_{24}H_{16}N_2,C_6H_3O_7N_3$ requires C, 54·7; H, 2·8%).

9-(4-*Cyano*-2-*nitrophenyl*)-1 : 2 : 3 : 4 : 10 : 11-*hexahydrocarbazole.*—1 : 2 : 3 : 4 : 10 : 11-Hexahydrocarbazole (17·5 g.) and 4-chloro-3-nitrobenzonitrile (18 g.), with anhydrous sodium acetate (10 g.) in alcohol (200 c.c.), were heated on a steam-bath for 10 hr. Red crystals separated and were collected from the cooled solution. After washing with dilute hydrochloric acid they were recrystallised from alcohol from which 9-(4-*cyano*-2-*nitrophenyl*)-1 : 2 : 3 : 4 : 10 : 11-*hexahydrocarbazole* (22 g.) separated as plates, m. p. 170—172° (Found: C, 71·2; H, 5·6. $C_{19}H_{17}O_2N_3$ requires C, 71·5; H, 5·4%).

9-(2-*Amino*-4-*cyanophenyl*)-1 : 2 : 3 : 4 : 10 : 11-*hexahydrocarbazole.*—A mixture of the above nitro-compound (12·8 g.), stannous chloride (46 g.), and glacial acetic acid saturated with hydrogen chloride (150 c.c.) was boiled for ½ hr. The green solution formed was treated with sodium hydroxide (200 g.) in water (1 l.) when it had cooled. The precipitate was collected and extracted with ether from which, after drying and evaporation, a greenish-yellow mass of

[7] Dunlop and Tucker, *J.*, 1939, 1945.

**9-(2-*amino*-4-*cyanophenyl*)-1 : 2 : 3 : 4 : 10 : 11-*hexahydrocarbazole*** (10—11 g.) was obtained. After two recrystallisation from alcohol this gave colourless needles, m. p. 176—177° (Found : C, 79·2; H, 6·8. $C_{19}H_{19}N_3$ requires C, 78·9; H, 6·6%).

**9-(2-*Amino*-4-*carboxyphenyl*)-1 : 2 : 3 : 4 : 10 : 11-*hexahydrocarbazole*.**—The solid which separated after the above amine had been boiled with hydrochloric acid (200 c.c.) for 2 hr. was extracted with sodium carbonate solution and, when this solution was brought to neutrality with dilute acetic acid, the free amino-acid was precipitated. This was dissolved in ether which was then dried and evaporated. The solid **9-(2-*amino*-4-*carboxyphenyl*)-1 : 2 : 3 : 4 : 10 : 11-*hexahydrocarbazole***, so obtained, was recrystallised from benzene from which it separated as needles, m. p. 199° (Found : C, 74·2; H, 6·5. $C_{19}H_{20}O_2N_2$ requires C, 74·0; H, 6·5%). This was converted into **9-(2-*acetamido*-4-*carboxyphenyl*)-1 : 2 : 3 : 4 : 10 : 11-*hexahydrocarbazole*** by warming it with a mixture of acetic acid and anhydride. The acetyl compound crystallised from benzene as plates, m. p. 211—214° (Found : C, 72·4; H, 6·5. $C_{21}H_{22}O_3N_2$ requires C, 72·0; H, 6·3%).

**9-(4-*Carboxy*-o-2′-*oxo*cyclo*hexylaminophenyl*)-1 : 2 : 3 : 4 : 10 : 11-*hexahydrocarbazole*.**—When the above amine (0·5 g.) was heated with 2-hydroxy*cyclo*hexanone (0·2 g.) at 120—140° water was evolved and the solid ***product*** crystallised from alcohol as needles, m. p. 189—191°, containing alcohol of crystallisation (Found : C, 71·0; H, 7·5; loss at 125° *in vacuo*, 10·4. $C_{35}H_{38}O_3N_2,C_2H_6O$ requires C, 72·0; H, 7·6; $C_2H_6O$, 10·2%). Reaction appeared to take place at 150—170° when this was heated with a drop of hydrochloric acid, but the product failed to crystallise.

**9-(4-*Cyano*-o-2′-*oxo*cyclo*hexylaminophenyl*)-1 : 2 : 3 : 4 : 10 : 11-*hexahydrocarbazole*** was prepared from the corresponding cyanide as above. Reaction took place at 120—130° in the presence of a little hydrochloric acid, and the product crystallised from alcohol as needles, m. p. 203—205° (Found : C, 78·0; H, 7·2. $C_{25}H_{27}ON_3$ requires C, 77·9; H, 7·0%). In some experiments when this product was heated to 180—200° with a little hydrochloric acid further loss of water took place and the mass crystallised when rubbed with alcohol. Recrystallisation from acetic acid afforded needles of **9-(5-*cyano*-1 : 2 : 3 : 4-*tetrahydro*-8-*carbazolyl*)-1 : 2 : 3 : 4 : 10 : 11-*hexahydrocarbazole***, m. p. 308—310° (with blackening) (Found : C, 78·3; H, 6·5. $C_{25}H_{25}N_3,\frac{1}{4}C_2H_4O_2$ requires C, 78·6; H, 6·8%). Acetic acid vapour could be detected when the substance was melted.

**9-*Nitroso*-1 : 9′-*dicarbazolyl*.**—1 : 9′-Dicarbazolyl (1·5 g.) in a little acetic acid was treated with sodium nitrite (0·3 g. in 3 c.c. of water). The yellow precipitate of **9-*nitroso*-1 : 9′-*dicarbazolyl*** was collected, washed, and dried. It had m. p. 155° (decomp.) (Found : N, 11·2. $C_{24}H_{15}ON_3$ requires N, 11·6%).

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## 234. *The Reaction between Diphenylpicrylhydrazyl and 2-Cyano-2-propyl Radicals.*

By J. C. BEVINGTON.

[$^{14}$C]Diphenylpicrylhydrazyl and [$^{14}$C]azo*iso*butyronitrile have been used in a study of the reaction between the hydrazyl and 2-cyano-2-propyl radicals. About 10% of the consumed hydrazyl is converted into the corresponding hydrazine. The products of the interaction of 2-cyano-2-propyl radicals are formed to some extent even if excess of hydrazyl is present in the solution; this may be due to decomposition of part of the azo-compound by a molecular mechanism rather than to a cage effect. Diphenylpicrylhydrazine also reacts with 2-cyano-2-propyl radicals. There is a rapid exchange of hydrogen atoms between diphenylpicrylhydrazyl and the corresponding hydrazine.

DIPHENYLPICRYLHYDRAZYL has been widely used as a scavenger for free radicals, but the chemical reactions involved are obscure. The simplest way in which the hydrazyl, itself a free radical, could capture another radical is by combining with it to give a tetrasubstituted hydrazine; there is however no evidence that a combination of this sort occurs to any appreciable extent.[1] In some cases the hydrazyl and the second radical might

[1] Wild, *Discuss. Faraday Soc.*, 1952, **12**, 127.

undergo disproportionation, to give diphenylpicrylhydrazine and an unsaturated product derived from the second radical. This might occur with the 2-cyano-2-propyl radicals generated in the decomposition of azo*iso*butyronitrile but would be most improbable with the phenyl or benzoate radicals derived from benzoyl peroxide. It is known that diphenylpicrylhydrazyl can abstract hydrogen atoms from certain molecules.[2]

Reagents labelled with $^{14}C$ have been used in a study of the chemistry of diphenylpicrylhydrazyl and results of the work are presented here. Although final conclusions concerning the mechanism of the scavenging action are not yet permissible, there is evidence that in some cases some of the hydrazyl is converted into the corresponding hydrazine. The labelled reagents have been used to effect accurate and sensitive analyses, by the isotope dilution technique, for the products of the interaction of the hydrazyl and radicals of known structure generated at known rates. The chemistry of diphenylpicrylhydrazine has also been studied.

## Experimental

The preparation and purification of most of the materials used have been described already.[3] [$^{14}C$]Diphenylpicrylhydrazyl was prepared from uniformly labelled picryl chloride by Goldschmidt and Renn's method,[4] and picryl chloride by standard methods from uniformly labelled aniline supplied by the Radiochemical Centre, Amersham. The labelling of the hydrazyl was confined to its picryl group. The hydrazyl was finally recrystallized from chloroform–ether. Diphenylpicrylhydrazine was recrystallized from ethyl acetate.

Unless stated otherwise, reactions were carried out in sealed tubes in the complete absence of air. For analyses by isotope dilution, the mixtures were diluted with carrier material in solution; the solvents were removed by distillation at low temperature. The procedures for *iso*butyronitrile and tetramethylsuccinodinitrile have been described already.[3] Diphenylpicrylhydrazine was purified by repeated crystallization from ethyl acetate; its activity reached a steady level after two crystallizations; in routine experiments the hydrazine was recrystallized three times before assay.

All assays were performed by gas counting. In the experimental results, the counting rates are quoted as counts per min. (c/m) for a fixed weight of carbon dioxide. These counting rates are directly proportional to the $^{14}C$ : $^{12}C$ ratio in the specimen.

Diphenylpicrylhydrazyl forms a complex with chloroform, and the material[5] crystallized from chloroform–ether has the formula $(C_{18}H_{13}O_6N_5)_2.CHCl_3$. The weights of hydrazyl used in various experiments have been corrected for the chloroform content; the counting rate for the labelled material has been corrected also.

## Results

By using [$^{14}C$]azo*iso*butyronitrile, it has been shown that when the azo-compound is decomposed in the presence of excess of diphenylpicrylhydrazyl significant quantities of tetramethylsuccinodinitrile are formed;[6] a small quantity of *iso*butyronitrile is also produced. The experiments are summarized in Table 1. The weights of tetramethylsuccinodinitrile and *iso*butyronitrile expected in experiments not involving the hydrazyl are quoted from previous work.[3]

Table 1.

| Expt. no. | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| $(CN\cdot CMe_2\cdot CN)_2$ (mg.) | 3·2 | 3·05 | 5·6 | 2·7 | 5·9 |
| $NPh_2\cdot NPc^*$ (mg.) | 6·6 | 13·1 | 36·9 | 35·7 | 30·4 |
| $C_6H_6$ (c.c.) | 1·6 | 1·5 | 2·8 | 1·35 | 2·0 |
| Time at 60° (hr.) | 16 | 16 | 16 | 16 | 16 |
| $(\cdot CMe_2\cdot CN)_2$ (mg.): found | 0·13 | 0·22 | 0·26 | 0·15 | † |
| " : expected in absence of $NPh_2\cdot NPc^*$ | 0·58 | 0·52 | 0·95 | 0·46 | 1·00 |
| $Pr^iCN$ (mg.): found | † | † | † | † | 0·005 |
| " : expected in absence of $NPh_2\cdot NPc^*$ | 0·26 | 0·24 | 0·45 | 0·22 | 0·47 |

* Pc = picryl. † Not determined.

[2] Braude, Brooke, and Linstead, *J.*, 1954, 3574.
[3] Bevington, *J.*, 1954, 3707.
[4] Goldschmidt and Renn, *Ber.*, 1922, **55**, 628.
[5] Lyons and Watson, *J. Polymer Sci.*, 1955, **18**, 141.
[6] Bevington, *Nature*, 1955, **175**, 477.

Similar experiments were done with diphenylpicrylhydrazine in place of the hydrazyl; the hydrazine interferes in the decomposition of the azo-compound. The results are summarized in Table 2.

TABLE 2.

| Expt. no. | 6 | 7 | 8 | 9 |
|---|---|---|---|---|
| $(\cdot NCMe_2 \cdot CN)_2$ (mg.) | 6·5 | 12·1 | 6·7 | 3·5 |
| $NPh_2 \cdot NHPc$ (mg.) | 31·1 | 73·8 | 41·5 | 21·1 |
| $C_6H_6$ (c.c.) | 1·6 | 2·75 | 1·5 | 1·0 |
| Time at 60° (hr.) | 4 | 6 | 24·5 | 78·5 |
| $(\cdot CMe_2 \cdot CN)_2$ (mg.) : found | 0·14 | † | 1·00 | 0·79 |
| ,, : expected in absence of $NPh_2 \cdot NHPc$ | 0·15 | 0·54 | 1·49 | 1·29 |
| $Pr^iCN$ (mg.) : found | 0·10 | 0·18 | 0·09 | 0·13 |
| ,, : expected in absence of $NPh_2 \cdot NHPc$ | 0·13 | 0·54 | 0·50 | 0·22 |

As will be shown, it is possible to formulate a mechanism by which the hydrazine could interfere in the decomposition of the azo-compound and then by a subsequent reaction be regenerated. Expt. 10 was designed to see if the hydrazine is consumed during experiments such as those of Table 2. 4·2 mg. of unlabelled azo-compound and 27·6 mg. of [$^{14}$C]diphenylpicrylhydrazine in 1·05 c.c. of benzene were kept at 60° for 24½ hr. (the conditions resemble those of expt. 8); analysis for the hydrazine at the end of the reaction showed that 22·5 mg. remained and that therefore 5·1 mg. had been consumed.

If there is an exchange of hydrogen between diphenylpicrylhydrazine and the corresponding hydrazyl, isotope dilution analysis cannot be applied, without modification, to the determination of small quantities of the hydrazine formed from the labelled hydrazyl. To test this, small weights of labelled hydrazyl were mixed in solution with larger weights of the unlabelled hydrazine: small samples of pure diphenylpicrylhydrazine were then isolated and assayed. In all cases the hydrazine was radioactive and its specific activity was nearly that expected for complete exchange between the hydrazyl and the hydrazine (Table 3).

TABLE 3.

| Expt. no. | 11 | 12 | 13 |
|---|---|---|---|
| [$^{14}$C]$NPh_2 \cdot NPc$ (mg.) | 5·55 | 5·28 | 14·20 |
| [$^{12}$C]$NPh_2 \cdot NHPc$ (mg.) | 312·4 | 437·9 | 493·0 |
| Solvent (c.c.) | 5·0 (E) | 2·0 (B) | 10·0 (C) |
| Calc. c/m for complete exchange | 989 | 683 | 1580 |
| Obs. c/m for $NPh_2 \cdot NHPc$ | 870 | 612 | 1540 |

E = ethyl acetate; B = benzene; C = chloroform.

In expt. 11, the solution of hydrazyl and hydrazine was kept at 60° for 23½ hr. in absence of air; in expt. 12 the air-free solution of the hydrazyl was kept at 60° for 40 hr. and then mixed with a solution of the hydrazine; in expt. 13 the solution of hydrazyl and hydrazine was kept at room temperature in the presence of air for 30 hr. before the hydrazine was recovered.

In spite of the rapid exchange, it is possible to use the labelled hydrazyl to see if the hydrazine is formed during the scavenging process. In expt. 14 and 15, 2-cyano-2-propyl radicals were generated from azo*iso*butyronitrile in the presence of excess of diphenylpicrylhydrazyl; isotope dilution analyses for the corresponding hydrazine were performed (Table 4).

TABLE 4.

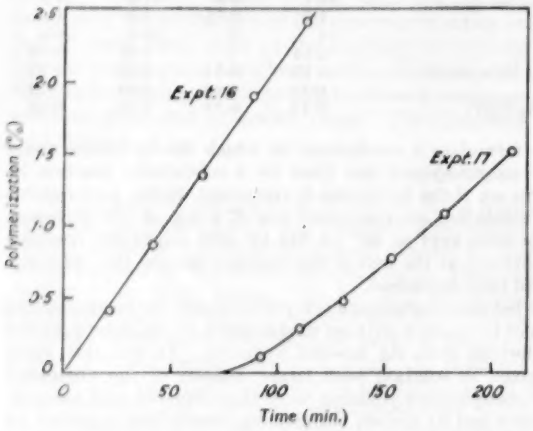
| Expt. no. | 14 | 15 | Expt. no. | 14 | 15 |
|---|---|---|---|---|---|
| $(\cdot NCMe_2 \cdot CN)_2$ (mg.) | 6·8 | 7·8 | Time at 60° (hr.) | 23·5 | 16·0 |
| [$^{14}$C]$NPh_2 \cdot NPc$ (mg.) | 28·6 | 23·5 | Carrier $NPh_2 \cdot NHPc$ (mg.) | 655·4 | 821·0 |
| $C_6H_6$ (c.c.) | 3·0 | 3·0 | c/m for purified $NPh_2 \cdot NHPc$ | 1050 | 665 |

Diphenylpicrylhydrazyl is an inhibitor for radical polymerizations.[7] In expt. 16, an air-free solution of azo*iso*butyronitrile in pure styrene (0·296 g./l. at 60°) was kept at 60°; the course of polymerization was followed dilatometrically. There was no induction period and the steady rate of polymerization was 1·30%/hr. In expt. 17, styrene containing the same concentration of azo*iso*butyronitrile but also 0·067 g. of diphenylpicrylhydrazyl per l. was treated similarly. For about 75 min. there was no observable polymerization; at that point the purple colour had almost completely disappeared and polymerization started. The conversion–time curves

[7] Bartlett and Kwart, *J. Amer. Chem. Soc.*, 1950, **72**, 1051.

(Figure) show that the rate of polymerization in expt. 17 was considerably less than that in expt. 16.

In expt. 18, styrene containing 0·296 g. of azo*iso*butyronitrile and 0·090 g. of [$^{14}$C]diphenylpicrylhydrazyl (57,200 c/m) per l. was kept at 60° until the induction period was over and there had been about 2% of polymerization. The polymer was recovered by precipitation in methanol and purified by dissolution in benzene and reprecipitation in methanol. After drying, the




*Polymerizations of styrene at* 60°: *Expt.* 16, *diphenylpicrylhydrazyl absent*; *expt.* 17, *diphenylpicrylhydrazyl present.*

polymer was buff in colour and possessed a counting rate of 43 c/m. This activity indicates that in the polymer one picryl group is combined for about 3000 molecules of monomeric styrene.

## Discussion

The results in Table 1 show that the products of the direct interaction of 2-cyano-2-propyl radicals are formed in appreciable quantities even although excess of diphenylpicrylhydrazyl is present in the solution. This indicates that a cage-effect may be operating or that part of the azo-compound decomposes by a mechanism not involving the liberation of free radicals. The apparent failure of the scavenger to capture all the radicals which seem to be generated in the solution is not confined to the hydrazyl, similar behaviour being observed if the azo-compound is decomposed in styrene.[8] It is perhaps significant that the yield of *iso*butyronitrile is depressed to a greater extent than that of tetramethylsuccinodinitrile. This may indicate that the azo-compound can decompose in two distinct ways; in the first, 2-cyano-2-propyl radicals are generated and in the absence of scavenger they interact by combination or disproportionation, but if sufficient scavenger is present they are all fixed; in the second type of decomposition, the dinitrile is formed but little *iso*butyronitrile is produced and this type of decomposition is unaffected by scavenger.

It has been suggested [9] that, to some extent, the following reactions occur :

(i) $CMe_2(CN)\cdot + NPh_2{\cdot}N{\cdot}C_6H_2(NO_2)_3 \longrightarrow CH_2{:}CMe{\cdot}CN + NPh_2{\cdot}NH{\cdot}C_6H_2(NO_2)_3$

(ii) $NPh_2{\cdot}NH{\cdot}C_6H_2(NO_2)_3 + CMe_2(CN)\cdot \longrightarrow NPh_2{\cdot}N{\cdot}C_6H_2(NO_2)_3 + CHMe_2{\cdot}CN$

This pair of reactions would allow for the regeneration of the hydrazyl and could account for the fact that the rate of production of radicals from azo*iso*butyronitrile is greater than the rate of removal of the hydrazyl, all the decomposed azo-compound being assumed to yield free radicals. If these reactions occur, significant quantities of *iso*butyronitrile should be formed when the azo-compound is decomposed in the presence of diphenylpicrylhydrazyl or its corresponding hydrazine, but expts. 5, 6, 7, 8, and 9 show conclusively

[8] Bevington, *Trans. Faraday Soc.*, 1955, **51**, 1392.
[9] Hammond, Sen, and Boozer, *J. Amer. Chem. Soc.*, 1955, **77**, 3244.

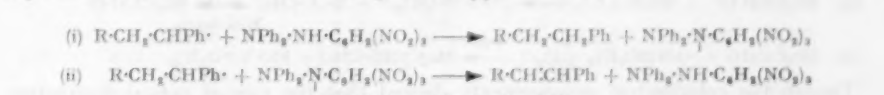
that this is not the case; further, if reactions (i) and (ii) occur readily the diphenylpicrylhydrazine would not be consumed, but expt. 10 showed that this is not so.

The ready exchange between diphenylpicrylhydrazyl and the corresponding hydrazine complicates the analyses for the hydrazine. It can be shown however that a small amount of the hydrazine is formed by the reaction of the hydrazyl with 2-cyano-2-propyl radicals. For, in expt. 14, if there had been complete exchange between the whole of the labelled hydrazyl present originally and the carrier diphenylpicrylhydrazine, the counting rate for the hydrazine would have been 2400 c/m. In fact some of the hydrazyl was consumed but if all the consumed material had changed into the hydrazine and then the residual hydrazyl had undergone exchange with the carrier, the counting rate for the diphenylpicrylhydrazine would still have been 2400 c/m. That the activity of the hydrazine was considerably less than that calculated for complete exchange shows that, of the hydrazyl which was consumed, a considerable part was not converted into the hydrazine. If the activity in the diphenylpicrylhydrazine was due solely to exchange between the residual hydrazyl and the carrier hydrazine, *i.e.*, no hydrazine was formed from the hydrazyl during the reaction, the weight of unconsumed hydrazyl can be calculated from the weight of carrier and the counting rates of the original [$^{14}$C]hydrazyl and the isolated diphenylpicrylhydrazine. For expt. 14, the weight of unconsumed hydrazyl is calculated as 12·2 mg. and the weight consumed as 16·4. mg. From the results of Bawn and Mellish,[10] the weight of azo*iso*butyronitrile which decomposed to radicals which reacted with diphenylpicrylhydrazyl was 3·9 mg.; if one $CMe_2(CN)\cdot$ radical is equivalent to one hydrazyl molecule, this weight of azo-compound is equivalent to 18·7 mg. of the hydrazyl. The discrepancy between the two values for the weight of hydrazyl consumed suggests that some of the consumed material was converted into the hydrazine; it appears that about 12% of the consumed hydrazyl reacted in this way. The rate of consumption of the hydrazine (see expt. 10) is not sufficient to invalidate this conclusion. It is concluded that in expt. 15 about 8% of the consumed hydrazyl was converted into the hydrazine.

Diphenylpicrylhydrazyl is an inhibitor for the polymerization of styrene, and after the induction period the rate of polymerization is much less than that for a similar system free from the hydrazyl (cf. expts. 16 and 17). These observations show that during the induction period, when the hydrazyl is reacting with 2-cyano-2-propyl radicals, it is converted into a substance which reacts with radicals. This substance becomes incorporated in the polymer since the polymer is discoloured and possesses activity if labelled hydrazyl is used (expt. 18). An objection therefore to the use of diphenylpicrylhydrazyl as a radical scavenger in exact work is that the products of the scavenging action can themselves react with free radicals. This conclusion has also been reached by Bengough.[11]

If the induction period in expt. 17 is 75 min., 0·013 g. of the azo-compound decomposed to radicals which reacted with the hydrazyl; in this calculation 1 l. of solution is considered and the velocity constant for the first-order decomposition of the azo-compound is taken as $1{\cdot}0 \times 10^{-5}$ sec.$^{-1}$ from the work of Bawn and Mellish.[10] If one $CMe_2(CN)\cdot$ radical reacts with one molecule of the hydrazyl, 0·062 g. of the hydrazyl would have been consumed during the induction period. Actually 1 l. of the reaction mixture contained 0·067 g. of diphenylpicrylhydrazyl. The agreement between the two weights of the hydrazyl is good evidence that each molecule of the scavenger reacts with one radical during the induction period.

Diphenylpicrylhydrazine is a retarder for the sensitized polymerization of styrene.[12] A possible mechanism for the retarding action appears to be:



(i) $R{\cdot}CH_2{\cdot}CHPh\cdot + NPh_2{\cdot}NH{\cdot}C_6H_2(NO_2)_3 \longrightarrow R{\cdot}CH_2{\cdot}CH_2Ph + NPh_2{\cdot}\underset{|}{N}{\cdot}C_6H_2(NO_2)_3$

(ii) $R{\cdot}CH_2{\cdot}CHPh\cdot + NPh_2{\cdot}\underset{|}{N}{\cdot}C_6H_2(NO_2)_3 \longrightarrow R{\cdot}CH{:}CHPh + NPh_2{\cdot}NH{\cdot}C_6H_2(NO_2)_3$

where R = a polystyrene chain.

[10] Bawn and Mellish, *Trans. Faraday Soc.*, 1951, **47**, 1216.
[11] Bengough, *Chem. and Ind.*, 1955, 599.
[12] Bevington, Ghanem, and Melville, unpublished work.

This is similar to a scheme discussed already;[9] according to it, the hydrazine would not become incorporated into the polymer but the use of labelled diphenylpicrylhydrazine has shown that the hydrazine does become incorporated in the polymer.[12]

The author thanks Dr. N. A. Ghanem for preparing the labelled diphenylpicrylhydrazyl, and Mr. C. S. Brooks for experimental assistance. He also thanks Professor H. W. Melville, F.R.S., for his interest and advice.

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## 235. *The Oxidation of iso*Butyraldehyde with the Free Radical, $\cdot O\cdot N(SO_3K)_2$, *from Potassium Nitrosyldisulphonate.*

By G. D. ALLEN and WILLIAM A. WATERS.

The oxidation of *iso*butyraldehyde by the free radical $\cdot O\cdot N(SO_3K)_2$, like that of oxidations by other 1-electron abstracting agents, occurs by electron removal from the enol anion: α-hydroxy*iso*butyraldehyde is the first product.

POTASSIUM NITROSYLDISULPHONATE (Frémy's salt), which in aqueous solution is completely dissociated into the purple free radical $\cdot O\cdot N(SO_3K)_2$, has recently been used for the oxidation of phenols and aromatic amines,[1] but its reactions with aliphatic molecules do not appear to have been studied. Since it should be a 1-electron abstracting reagent it was expected that it would resemble potassium ferricyanide and oxidise aliphatic aldehydes and ketones in alkaline solution by attacking them in the forms of their enol anions.[2]

If carefully prepared, Frémy's salt gives deep purple solutions, reasonably stable[3] in the pH range 8—11, which can easily be analysed colorimetrically as their absorptions in the 545 mμ region are directly proportional to their concentrations as measured both by weighing of the orange dimeric solid and by iodine–thiosulphate titration. Solutions in which some decomposition has occurred can still be estimated colorimetrically, though not titrimetrically since hydrolysis products then interfere.

Preliminary tests showed that aliphatic aldehydes were oxidised at measurable speeds in alkaline carbonate solutions at 25° whereas oxidation of ketones and nitro-paraffins required more strongly alkaline conditions under which some self-decomposition of the purple free radical occurred. The kinetics of the oxidation of *iso*butyraldehyde have consequently been studied in carbonate–bicarbonate buffers. The experimental results, which are collected in the Tables and the Figure, show that in buffers of constant ionic strength the initial rate of decolorisation of the free radical is a first-order process with respect to the radical itself, to *iso*butyraldehyde, and, approximately, to hydroxyl ion. This oxidation occurs at about a tenth of the rate of oxidation of *iso*butyraldehyde by alkaline ferricyanide (which is a zero-order process with respect to the oxidant [2]) and it can therefore be concluded that the rate-determining stage is that of electron abstraction from the enol anion (reaction 2).

(1) $Me_2CH\cdot CHO + OH^- \underset{\text{maintained}}{\overset{\text{Equilm.}}{\rightleftharpoons}} Me_2\dot{C}H\cdot O^- + H_2O$

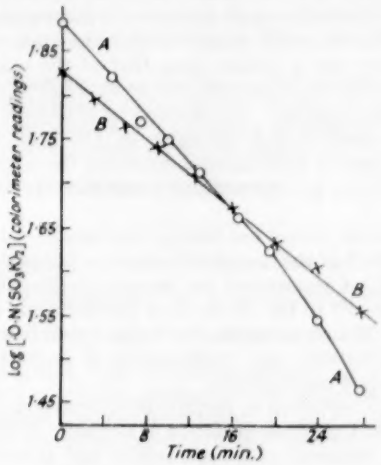
(2) $Me_2C{:}CH\cdot O^- + \cdot ON(SO_3K)_2 \xrightarrow{\text{Slow}} \{\dot{O}\cdot N(SO_3K)_2\}^- + Me_2\dot{C}\cdot CHO \longleftrightarrow Me_2C{:}CH\cdot O\cdot$ (Mesomeric)

(3) $Me_2\dot{C}\cdot CHO + \cdot O\cdot N(SO_3K)_2 + H_2O \xrightarrow{\text{Fast}} Me_2C(OH)\cdot CHO + HO\cdot N(SO_3K)_2$

Though the colorimetric measurements showed that the rate of radical destruction increased in the later stages of the oxidation (see Figure), as would be the case if the primary

[1] Teuber *et al.*, *Chem. Ber.*, 1952, **85**, 95; 1953, **86**, 1036; 1954, **87**, 1236, 1252.
[2] Speakman and Waters, *J.*, 1955, 40.
[3] Murib and Ritter, *J. Amer. Chem. Soc.*, 1952, **74**, 3394; Li and Ritter, *ibid.*, 1953, **75**, 3024, 5823, 5828, 5831.

product underwent further oxidation, it was possible to show that after reaction mixtures gave positive qualitative tests for α-hydroxy*iso*butyraldehyde.[4] From the mixture of 2 : 4-dinitrophenylhydrazones obtained from such oxidised solutions that of α-methylacraldehyde, $CH_2$:CMe·CHO, the dehydration product of α-hydroxy*iso*butyraldehyde, was




*A, No added salt.*
*B,* $NaClO_4$ *added.*

TABLE 1. *Oxidation of* iso*butyraldehyde with the radical* ·O·N(SO$_3$K)$_2$ *at 25° and* pH 9·63.

Initial aldehyde concn. = 6·44 × 10$^{-2}$M.

| Time (min.) | [Radical] ($10^{-2}$M) | $\log_{10}$ [Radical] | $10^2 k_{uni}$ | Time (min.) | [Radical] ($10^{-2}$M) | $\log_{10}$ [Radical] | $10^2 k_{uni}$ |
|---|---|---|---|---|---|---|---|
| 0·0 | 9·10 | $\bar{1}$·9590 | 3·11 | 24·0 | 3·93 | $\bar{1}$·5944 | 3·34 |
| 2·0 | 8·55 | $\bar{1}$·9320 | 3·73 | 27·5 | 3·60 | $\bar{1}$·5563 | 3·37 |
| 4·5 | 7·71 | $\bar{1}$·8871 | 2·97 | 31·5 | 3·20 | $\bar{1}$·5051 | 3·32 |
| 9·5 | 6·96 | $\bar{1}$·8426 | 3·29 | 34·3 | 2·80 | $\bar{1}$·4472 | 3·43 |
| 12·3 | 6·06 | $\bar{1}$·7825 | 3·34 | 41·3 | 2·53 | $\bar{1}$·4031 | 3·11 |
| 15·0 | 5·52 | $\bar{1}$·7419 | 3·35 | 44·3 | 2·28 | $\bar{1}$·3579 | 3·36 |
| 18·0 | 5·17 | $\bar{1}$·7139 | 3·13 | 47·3 | 1·73 | $\bar{1}$·2380 | 3·46 |
| 21·5 | 4·46 | $\bar{1}$·6493 | 3·32 | | | | |

Mean $k_{uni}$ = 3·31 × 10$^{-2}$ min.$^{-1}$.

TABLE 2. *Velocity of oxidation of* iso*butyraldehyde by the free radical* ·O·N(SO$_3$K)$_2$ *at 25°.*

| pH | [OH$^-$] ($10^{-6}$M) | $10^2 k_{uni}$ (min.$^{-1}$) | No. of obsns. | [Aldehyde] ($10^{-2}$M) | $k$/[Aldehyde] | Mean | $10^{-2}k$/[Ald.][OH$^-$] |
|---|---|---|---|---|---|---|---|
| 10·65 | 44·7 | 4·44 | 7 | 1·46 | 3·04 | | |
| | | 5·45 | 5 | 1·75 | 3·11 | 3·08 | 6·89 |
| | | 5·43 | 6 | 1·75 | 3·10 | | |
| 10·36 | 22·9 | 7·20 | 5 | 4·54 | 1·58 | 1·57 | 6·86 |
| | | 4·37 | 6 | 2·77 | 1·57 | | |
| 10·18 | 15·1 | 1·72 | 6 | 1·72 | 1·00 | | |
| | | 1·84 | 5 | 1·72 | 1·06 | 1·03 | 6·82 |
| | | 4·21 | 6 | 4·10 | 1·02 | | |
| 9·95 | 8·91 | 1·29 | 8 | 1·44 | 0·90 | | |
| | | 2·88 | 6 | 3·55 | 0·81 | 0·86 | 9·6 |
| | | 6·23 | 4 | 7·09 | 0·88 | | |
| | | 7·36 | 4 | 8·78 | 0·84 | | |
| 9·63 | 4·27 | 1·92 | 6 | 3·45 | 0·56 | | |
| | | 3·06 | 8 | 5·17 | 0·59 | 0·55 | 12·9 |
| | | 3·31 | 14 | 6·44 | 0·51 | | |
| | | 5·04 | 5 | 8·62 | 0·58 | | |

In all kinetic experiments the aldehyde concentration was greatly in excess of that of the free radical. Concentrations were measured for 50—70% disappearance of the latter.

[4] Conant and Aston, *J. Amer. Chem. Soc.*, 1928, **50**, 2783.

separated, confirming the view that the free-radical oxidation of aliphatic aldehydes occurs at the carbon atom adjacent to the aldehyde group.

The Figure shows that the rate of oxidation is significantly reduced by the addition of the neutral salt potassium perchlorate. Addition of sodium chloride produced a similar effect. Since the oxidation stages given above are reactions between ions of similar sign, $Me_2CH{:}CH{\cdot}O^-$ and $\{\cdot ON(SO_3)_2\}^{2-}$, or between anions and neutral molecules, with which a positive salt effect would be expected, we would point out that the ionic strength of the buffer solution (0·4M) was always very much greater than that of the reactants ($10^{-2}$ to $10^{-3}$M) and suggest that at this very high ionic strength the neutral salt may be acting by diminishing the overall basicity of the medium. Enolisation of aldehydes is, after all, a process controlled by general base catalysis and the value of $[OH^-]$ indicated by glass electrode measurements (always subject to instrumental errors on the alkaline side) may not be quite the true function for use in representing quantitatively the equilibrium represented by reaction (1) above.

Kinetic measurements have also been carried out with propaldehyde, with a similar but less decisive outcome. The initial stage of the oxidation is again a first-order process with respect to the $\cdot O\cdot N(SO_3K)_2$ radical, is accelerated by increasing the alkalinity of the medium, and is of first order with respect to the aldehyde at low aldehyde concentrations. The rate, however, increases above that required for first-order dependence both at high aldehyde and at high alkali concentrations, and examination of products showed that aldolisation products were being formed concurrently.

It is not proposed to examine this oxidation in further detail since the present work is sufficient to confirm the view that the 1-electron oxidation of aliphatic aldehydes regularly occurs *via* the enol so as to give a resonance-stabilised radical and therefrom a product oxidised in the α-position to the CHO group.

## Experimental

Potassium nitrosyl disulphonate was prepared as described by Palmer,[5] at <0° during the whole procedure, washed free from sulphite with 10% aqueous potassium hydroxide, then with anhydrous ethanol, and stored in a vacuum. Solid which had been kept for more than 3 days was discarded, and fresh aqueous solutions were prepared each day. Freshly prepared solid, when analysed by means of iodine–thiosulphate, had a purity of at least 99·7%. 0·05M-Solutions in carbonate–bicarbonate buffers changed very little in colour intensity during 50 hr. at room temperature. The colorimetric measurements were made with a "Spekker" photoelectric absorptiometer and light of about 550 mμ (yellow–green, no. 605, filters). Linear plots relating absorption to concentration were regularly obtained so that at the molarities used the dissociation of the orange dimeric salt to the purple $\cdot O\cdot N(SO_3K)_2$ free radical must be complete. To adjust changes in its sensitivity the absorptiometer was calibrated daily.

Before use the aldehydes were freshly distilled from iron wire in a current of nitrogen, and solutions were made up by weight in distilled water which had been flushed with nitrogen. Buffer solutions were prepared from "AnalaR" sodium carbonate and sodium hydrogen carbonate so as to have a constant ionic strength corresponding to that of 0·4M-sodium carbonate. pH Measurements were made with a Doran instrument and an "Alkacid" glass electrode.

For kinetic measurements solid Frémy's salt was dissolved in the requisite buffer solutions, flushed with nitrogen, brought to the requisite temperature, and then mixed in the thermostat with due proportions of the air-free aldehyde solution. A small quantity of each mixture was transferred to a bubbler, and pure nitrogen was slowly passed through this and then through the main bulk of the reactants so that loss of aldehyde as vapour from the reacting mixture was prevented. At intervals samples of the reaction mixtures were pipetted into the optical cells which already contained a known volume of concentrated sodium hydrogen carbonate solution, sufficient to reduce the alkalinity to such an extent that further reaction ceased.

*Investigation of Reaction Products from* iso*Butyraldehyde.*—(*a*) The colourless oxidised solutions were shown to contain free potassium hydroxylaminedisulphonate, $HO\cdot N(SO_3K)_2$, since treatment with lead dioxide re-formed the purple radical.[3] (*b*) The colourless solution did not give an iodoform reaction until it had been boiled with 30% aqueous sodium hydroxide for

[5] Palmer, "Experimental Inorganic Chemistry," Cambridge Univ. Press, 1954.

30 min. This is regarded by Conant and Aston [4] as diagnostic for the presence of α-hydroxy*iso*butyraldehyde. (*c*) Warming an oxidised solution with 2 : 4-dinitrophenylhydrazine in hydrochloric acid gave a mixture of orange dinitrophenylhydrazones. These were collected, dissolved in carbon tetrachloride, and passed through acid-washed alumina. By crystallisation, from ethanol, of the poorly separated fractions there were obtained the dinitrophenylhydrazones of *iso*butyraldehyde, m. p. 182°, acetone, m. p. and mixed m. p. 126°, and α-methylacraldehyde, m. p. 204° (Found : C, 48·1; H, 4·0; N, 22·4. Calc. for $C_{10}H_{10}O_4N_4$ : C, 48·0; H, 4·0; N, 22·4%).

The measurements shown in the Figure were carried out at pH 9·63 with a buffer containing 16·8 g. of sodium hydrogen carbonate and 39·75 g. of sodium carbonate per l. Both reaction mixtures contained the same initial quantity of aldehyde, but to mixture B there were also added (per l.) 60 g. of sodium perchlorate monohydrate.

THE DYSON PERRINS LABORATORY, OXFORD. [*Received, September 19th*, 1955.]

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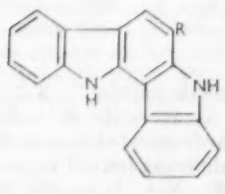
## 236. *Experiments on the Preparation of Indolocarbazoles. Part VIII.* The Preparation of* 1-*Methylindolo*(2′ : 3′-2 : 3)*carbazole.*

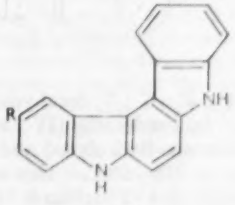
By MARGARET L. SWINDELLS and MURIEL L. TOMLINSON.

The preparation of 1-methylindolo(2′ : 3′-2 : 3)carbazole from 7-amino-1 : 2 : 3 : 4 : 10 : 11-hexahydro-8-methylcarbazole and 2-hydroxy*cyclo*hexanone is described. It has been shown to be very difficult to acylate 8-chloro-1 : 2 : 3 : 4-tetrahydrocarbazole and the corresponding 8-methyl compound.

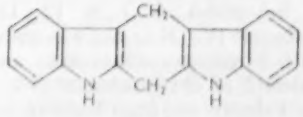
THERE are five possible isomeric indolocarbazoles and the literature appears to contain references to simple derivatives of only two of them. Indolo(3′ : 2′-1 : 2)carbazole [1] (I; R = H) and its 3-methyl derivative (I; R = Me[2]) and 6-cyanoindolo(2′ : 3′-3 : 4)carbazole [3] (II; R = CN) have been described. Dobeneck and Maas [4] recently prepared a compound $C_{18}H_{14}N_2$, which they formulated as (III).† This was obtained, in small yield, as a




(I)




(II)




(III)

sparingly soluble yellow compound, m. p. 408°, from indole or 3 : 3′-di-indolylmethane and formaldehyde in the presence of acid. We have failed to obtain this compound from indole but, if the constitution allotted to it is correct, it should give, on dehydrogenation, the parent of the methylindolocarbazole described below.

It has been shown that aromatic amines can often be conveniently converted into tetrahydrocarbazoles by condensation with 2-hydroxy*cyclo*hexanone [5,6,7] and we decided to investigate the preparation of indolo(2′ : 3′-2 : 3)carbazoles from 7-amino-1 : 2 : 3 : 4-tetrahydro- or 7-amino-1 : 2 : 3 : 4 : 10 : 11-hexahydro-carbazoles by this route. This

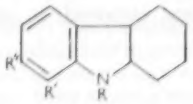
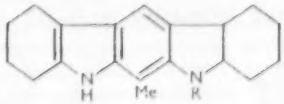
* Part VII, *J.*, 1955, 337.

† The authors named this 5 : 6 : 7 : 12-tetrahydroindolo[2′ : 3′-*b*]carbazole in accordance with the practice of *Chemical Abstracts*.

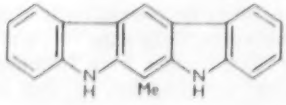
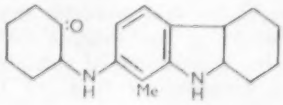
[1] Tomlinson, *J.*, 1951, 809.
[2] Hall and Plant, *J.*, 1953, 116.
[3] Clifton and Plant, *J.*, 1951, 461.
[4] Dobeneck and Maas, *Chem. Ber.*, 1954, **87**, 455.
[5] Jones and Tomlinson, *J.*, 1953, 4114.
[6] Carter, Katritzky, and Plant, *J.*, 1955, 337.
[7] Cummins and Tomlinson, *J.*, 1955, 3475.

will be possible only if the 8-position is blocked, as otherwise ring closure will take place there preferentially and give the "angular" isomer. We did not expect that suitable tetrahydrocarbazoles could be directly nitrated in the 7-position, and indeed 8-chloro-1 : 2 : 3 : 4-tetrahydrocarbazole yielded a yellow amorphous product which may be an *N*-nitro-compound, but it was hoped that 9-acetyl-8-chloro-1 : 2 : 3 : 4-tetrahydrocarbazole and the corresponding 8-methyl compound would be nitrated in the 7-position. It proved extremely difficult to prepare these compounds: all the normal methods of acetylation and benzoylation failed. A small yield of 9-acetyl-8-chloro-1 : 2 : 3 : 4-tetrahydrocarbazole was obtained by treating 8-chloro-1 : 2 : 3 : 4-tetrahydrocarbazolylmagnesium bromide with acetyl chloride but even this method failed with the 8-methyl compound although the indole seemed to react with ethylmagnesium bromide. Further, although 9-acetyl-1 : 2 : 3 : 4-tetrahydrocarbazole affords the 7-nitro-compound readily,[8,9] nitration of this 8-chloro-compound, under a variety of conditions, either converted it into a tar or left it unchanged.

It is known that 1 : 2 : 3 : 4 : 10 : 11-hexahydrocarbazole is nitrated in the 7-position in concentrated sulphuric acid,[9,10] and it seemed likely that nitro-compounds suitable for our purpose could be made by a similar method although an *ortho*- and *para*-directing group in the 8-position might make the nitro-group enter the 5-position instead. 1 : 2 : 3 : 4 : 10 : 11-Hexahydro-8-methylcarbazole (IV; R = R″ = H, R′ = Me) was obtained in good yield by reducing the tetrahydro-compound with tin and hydrochloric acid, but the chloro-compound (IV; R = R″ = H, R′ = Cl) was obtained only in yields too small to be useful. Nitration proceeded as expected to give the product (IV; R = H, R′ = Me, R″ = $NO_2$) and a compound which is most likely (IV; R = H, R′ = Cl, R″ = $NO_2$). Reduction of both the compounds (IV; R = H, R′ = Me, R″ = $NO_2$) and (IV; R = Ac, R′ = Me, R″ = $NO_2$) gave primary amines which were not solid at room temperature, but both amines afforded the same solid diacetyl compound (IV; R = Ac, R′ = Me, R″ = NHAc).

(IV)   (V)

9-Acetyl-5 : 6 : 7 : 8 : 12 : 13 : 4′ : 5′ : 6′ : 7′-decahydro-1-methylindolo(2′ : 3′-2 : 3)-carbazole (V; R = Ac) was obtained by condensing (IV; R = Ac, R′ = Me, R″ = $NH_2$) with 2-hydroxy*cyclo*hexanone and this was hydrolysed and dehydrogenated to 1-methylindolo(2′ : 3′-2 : 3)carbazole (VI); m. p. 278—280°. This was different (mixed m. p. and light absorption) from 3-methylindolo(3′ : 2′-1 : 2)carbazole [2] (I; R = Me). It was also found that the secondary amino-group in the compound (IV; R = H, R′ = Me, R″ = $NH_2$) did not interfere with condensation with 2-hydroxy*cyclo*hexanone. They reacted to give 1 : 2 : 3 : 4 : 10 : 11-hexahydro-8-methyl-7-2′-oxo*cyclo*hexylaminocarbazole (VII), which was dehydrated and dehydrogenated to give the previous material (VI).

(VI)   (VII)

That the linear compound (VI), and not (I), was obtained from the aminohexahydrocarbazole employed proves that 1 : 2 : 3 : 4 : 10 : 11-hexahydro-8-methylcarbazole is nitrated in the 7-position in sulphuric acid.

[8] Perkin and Plant, *J.*, 1921, **119**, 1825; *J.*, 1923, **123**, 676.
[9] Plant, *J.*, 1936, 899.
[10] Gurney and Plant, *J.*, 1927, 1316.

## Experimental

*9-Acetyl-8-chloro-1 : 2 : 3 : 4-tetrahydrocarbazole.*—8-Chloro-1 : 2 : 3 : 4-tetrahydrocarbazole (5·8 g.) in ether, was added gradually to a solution of ethylmagnesium bromide, made by dissolving magnesium (0·8 g.) in ethyl bromide (4·7 g.) and ether (100 c.c.). The yellow solution formed was boiled for 5 min. and slowly treated with acetyl chloride (2·7 g.) in ether. After 1 hr. dilute sulphuric acid was added and the ethereal layer was separated, washed, and dried. After the solvent had been evaporated the residue was distilled and collected in two fractions: that having b. p. 145—155°/0·1 mm. was unchanged chlorotetrahydrocarbazole; that having b. p. 155—165°/0·1 mm. was recrystallised from methanol from which *9-acetyl-8-chloro-1 : 2 : 3 : 4-tetrahydrocarbazole* separated as plates, m. p. 85° raised to 88° by further recrystallisation (Found: C, 67·5; H, 5·8. $C_{14}H_{14}ONCl$ requires C, 67·9; H, 5·7%). The acetyl group could be removed by hydrolysis with aqueous-alcoholic potassium hydroxide.

*1 : 2 : 3 : 4 : 10 : 11-Hexahydro-8-methylcarbazole.*—1 : 2 : 3 : 4-Tetrahydro-8-methylcarbazole (86 g.), hydrochloric acid (160 c.c.), ethanol (160 c.c.), and tin (160 g.) were heated together on a steam-bath for 4—5 hr. When cold, the solution thus obtained was decanted from the tin and alcohol was removed by steam-distillation. The resulting solution, containing the precipitated stannichloride of the required base, was treated with 40% sodium hydroxide solution (800 c.c.), and *1 : 2 : 3 : 4 : 10 : 11-hexahydro-8-methylcarbazole* was steam-distilled, collected, and recrystallised from aqueous alcohol (75%) from which it separared as needles, m. p. 48°, containing solvent (50 g.). After drying at 70° (loss, 13·8%) it was obtained as a viscous liquid (Found: C, 83·5; H, 9·1. $C_{13}H_{17}N$ requires C, 83·4; H, 9·2%). The *hydrochloride* crystallised from dilute hydrochloric acid as prisms, m. p. 216° (Found: Cl, 15·9. $C_{13}H_{17}N,HCl$ requires Cl, 15·8%). Treatment with acetic anhydride at 100° for 10 min. afforded *9-acetyl-1 : 2 : 3 : 4 : 10 : 11-hexahydro-8-methylcarbazole* which separated from ethanol as plates, m. p. 89° (Found: C, 78·4; H, 8·3. $C_{15}H_{19}ON$ requires C, 78·6; H, 8·3%). Benzoylation (Schotten–Baumann) gave the *9-benzoyl* derivative as needles (from ethanol), m. p. 114° (Found: C, 82·5; H, 7·4. $C_{20}H_{21}ON$ requires C, 82·4; H, 7·3%).

*8-Chloro-1 : 2 : 3 : 4 : 10 : 11-hexahydrocarbazole.*—This was prepared in a similar manner from the chlorotetrahydrocarbazole (24 g.), hydrochloric acid (40 c.c.), ethanol (40 c.c.), and tin (40 g.). The mixture was vigorously stirred and boiled for 4—5 hr. Hydrochloric acid (20 c.c.) and ethanol (20 c.c.) were then added and at 2-hourly intervals two further similar additions were made before removing alcohol and making the residue alkaline. The product coagulated in the alkaline solution and was removed (by decanting the liquid), dissolved in ether, washed, and dried ($MgSO_4$). After removal of the ether the basic hexahydrocarbazole was extracted from unchanged starting material with dilute hydrochloric acid, liberated with alkali, and re-extracted with ether. When the dried solvent had been removed again, 8-chloro-1 : 2 : 3 : 4 : 10 : 11-hexahydrocarbazole (2 g.) remained as a colourless liquid that did not solidify. The *9-acetyl derivative* separated from aqueous ethanol (70%) as needles, m. p. 78·5—79·5° (Found: C, 67·5; H, 6·5. $C_{14}H_{16}ONCl$ requires C, 67·3; H, 6·5%). Its *hydrochloride* separated from dilute hydrochloric acid as prisms, m. p. 199—206° (decomp.) (Found: C, 59·4; H, 6·5. $C_{12}H_{14}NCl,HCl$ requires C, 59·0; H, 6·2%), and its *picrate* from ethanol as yellow prisms, m. p. 144° (Found: C, 49·7; H, 4·1. $C_{12}H_{14}NCl,C_6H_2O_7N_3$ requires C, 49·5; H, 3·9%).

*1 : 2 : 3 : 4 : 10 : 11-Hexahydro-8-methyl-7-nitrocarbazole.*—The above hexahydromethylcarbazole (10·8 g.) in sulphuric acid (100 c.c.) was treated at 3° with potassium nitrate (5·8 g.). After 15 min. the solution was poured on ice and neutralised at 0° with aqueous ammonia (*d* 0·88). The product was collected with ether and dried ($MgSO_4$), and, when the solvent had been evaporated, the residue solidified and was recrystallised from aqueous ethanol (75%) from which *1 : 2 : 3 : 4 : 10 : 11-hexahydro-8-methyl-7-nitrocarbazole* separated as yellow plates, m. p. 67—70° (9·6 g.), raised to 71·5—72·5° by further recrystallisation( Found: C, 67·5; H, 7·1. $C_{13}H_{16}O_2N_2$ requires C, 67·2; H, 6·9%). The above product, m. p. 67—70° (3 g.), in light petroleum (b. p. 60—80°), was run on to a column of activated alumina and eluted with solvent containing increasing proportions of benzene, and finally with benzene. Many fractions were collected and separately evaporated. Mixed m. p. determinations with the solids obtained showed that the material was homogeneous and therefore that there was no 5-nitro-compound present. *9-Acetyl-1 : 2 : 3 : 4 : 10 : 11-hexahydro-8-methyl-7-nitrocarbazole*, made by treatment with acetic anhydride at 100°, separated from aqueous ethanol (50%) as prisms, m. p. 178—179° (Found: C, 65·7; H, 6·7. $C_{15}H_{18}O_3N_2$ requires C, 65·7; H, 6·6%).

*8-Chloro-1 : 2 : 3 : 4 : 10 : 11-hexahydro-7-nitrocarbazole* was prepared in a similar way.

It crystallised from ethanol (75%) as golden needles, m. p. 158—159° (Found, in a sample dried at 130° : C, 57·2; H, 5·4. $C_{12}H_{13}O_2N_2Cl$ requires C, 57·1; H, 5·2%).

*7-Acetamido-9-acetyl-*1 : 2 : 3 : 4 : 10 : 11-*hexahydro-8-methylcarbazole.*—(i) The methylnitro-compound above (1·5 g.) and Adams catalyst (0·01 g.) were shaken in methanol (75 c.c.) in contact with hydrogen at N.T.P. The colour disappeared and hydrogen (435 c.c.) was absorbed. When the catalyst and solvent had been removed, 7-amino-1 : 2 : 3 : 4 : 10 : 11-hexahydro-8-methylcarbazole remained as a liquid that solidified to a glass at 0°. Recrystallisation from light petroleum (b. p. 100—120°) gave light brown prisms, m. p. 125—135° (decomp.), containing solvent which could be removed *in vacuo*, leaving the base as a liquid again. Treatment with acetic anhydride at 100° for 10 min. followed by addition of water gave *7-acetamido-9-acetyl-*1 : 2 : 3 : 4 : 10 : 11-*hexahydro-8-methylcarbazole* as prisms, m. p. 203—204° (Found : C, 70·9; H, 7·8. $C_{17}H_{22}O_2N_2$ requires C, 71·3; H, 7·7%). (ii) Similar reduction of 9-acetyl-1 : 2 : 3 : 4 : 10 : 11-hexahydro-8-methyl-7-nitrocarbazole also gave the corresponding amine as a glass, and acetylation converted this into the diacetyl compound described above.

*9-Acetyl-*5 : 6 : 7 : 8 : 12 : 13 : 4′ : 5′ : 6′ : 7′-*decahydro-*1′-*methylindolo*(2′ : 3′-2 : 3)-*carbazole.*—The above 9-acetyl-7-aminohexahydrocarbazole (1·5 g.) was heated with 2-hydroxy*cyclo*hexanone (0·7 g.) at 120—130° until evolution of water ceased. Hydrochloric acid (1 drop) was then added and the temperature was raised to 135—145°; further reaction occurred and the solid formed crystallised in contact with alcohol (40—50 c.c.). Recrystallisation from 90% acetic acid gave *9-acetyl-*5 : 6 : 7 : 8 : 12 : 13 : 4′ : 5′ : 6′ : 7′-*decahydro-*1-*methylindolo*(2′ : 3′-2 : 3)-*carbazole* as prisms, m. p. 284—285° (1·3 g.) (Found : C, 77·9; H, 8·1. $C_{21}H_{26}ON_2$ requires C, 78·2; H, 8·1%).

1-*Methylindolo*(2′ : 3′-2 : 3)*carbazole.*—(i) The above acetyldecahydro-compound (0·5 g.) was boiled for 20 min. with sulphuric acid (10 c.c.) and water (10 c.c.). The solution was cooled and neutralised with ammonia; the dark green powder (0·4 g. after drying) that was collected could not be crystallised. It was heated with palladium–charcoal (0·4 g. containing 10% of palladium) in an atmosphere of carbon dioxide at 320—330° for 1½ hr. The resulting mass was extracted with acetone (50 c.c.), and dilution with water gave a solid, m. p. 268° (0·2 g.). It was sublimed *in vacuo*, giving greenish-yellow needles of 1-*methylindolo*(2′ : 3′-2 : 3)*carbazole*, m. p. 278° raised to 278—280° by recrystallisation from benzene from which it separated as colourless needles, which showed a brilliant green fluorescence in ultraviolet light (Found : C, 84·3; H, 5·3 . $C_{19}H_{14}N_2$ requires C, 84·4; H, 5·2%). The m. p. was depressed to 245—250° on admixture with 3-methylindolo(3′ : 2′-1 : 2)carbazole, m. p. 257°, prepared by dehydrogenation of 3-methyl-5 : 6 : 7 : 8 : 4′ : 5′ : 6′ : 7′-octahydroindolo(3′ : 2′-1 : 2)carbazole (see Jones and Tomlinson [5] and Hall and Plant [2] who gave m. p. 261—262°).

(ii) Condensation of 7-amino-1 : 2 : 3 : 4 : 10 : 11-hexahydro-8-methylcarbazole (0·7 g.) with 2-hydroxy*cyclo*hexanone (0·4 g.) and a little hydrochloric acid at 120—130° gave a product which crystallised when triturated with acetone and was obtained as prisms, m. p. 290—300° (decomp.), from 90% acetic acid (Found : C, 70·5; H, 7·9. $C_{19}H_{26}ON_2,C_2H_4O_2$ requires C, 70·3; H, 8·4%). This therefore appears to be 1 : 2 : 3 : 4 : 10 : 11-*hexahydro-8-methyl-7-*2′-*oxo*cyclo*hexylaminocarbazole acetate.* It was not possible to isolate the decahydroindolocarbazole by further heating of this compound but, when it (0·4 g.) was treated with palladium–charcoal (0·4 g.) as above, 1-methylindolo(2′ : 3′-2 : 3)carbazole, m. p. 278° (identical with the above), was isolated. Light absorption (log ε in parentheses) : $\lambda_{max.}$ 2400 (4·614), 2650 (4·547), 2750 (4·538), 3060 (4·794), 3440 (4·055), 3600 (4·131) Å; to be compared with $\lambda_{max.}$ 2460 (4·590), 2650 (4·607), 2870 (4·516), 3200 (4·053), 3430 (4·006), 3580 (4·187) Å for the isomeric 3-methylindolo(3′ : 2′-1 : 2)carbazole. It dissolved in sulphuric acid forming a violet solution, changed to bluish-green fading to reddish-brown on addition of a little nitric acid. It was unaffected by boiling it with acetic anhydride and charred if a little sulphuric acid was added to this solution.

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## 237. *Structural Chemistry of the Alkoxides. Part V.* Isomeric Butoxides and Pentyloxides of Quinquevalent Tantalum.*

By D. C. Bradley, W. Wardlaw, and (Miss) A. Whitley.

The isomeric butoxides $Ta(O\cdot C_4H_9)_5$ and the isomeric pentyloxides $Ta(O\cdot C_5H_{11})_5$ of tantalum have been prepared by various methods. Boiling points under reduced pressure, and molecular weights, were determined and the results are discussed in terms of the steric theory for metal alkoxides.

We have already reported [1] that tantalum penta-*n*-alkoxides, $Ta(O\cdot C_nH_{2n+1})_5$ where $n$ = 1—4, are dimeric in boiling benzene. In other solvents, such as toluene, pyridine, acetonitrile, or di*iso*propyl ether, different molecular complexities were found [2] but the value in each solvent was practically constant for the series of alkoxides. Thus it appeared that the chain length of the normal alkyl group had a negligible effect on the molecular complexity of the alkoxide. As a consequence of our theory [3] developed to explain the properties of the tetra-alkoxides of Group IVA metals, it was believed that the molecular complexity and volatility of tantalum penta-alkoxides would be strongly dependent on the degree of branching of the alkyl group. To test this and to extend our knowledge of tantalum alkoxides the new isomeric butoxides and isomeric pentyloxides were prepared and their molecular weights and volatilities determined. The phenomenon of alcohol interchange involving tantalum alkoxides was demonstrated by the preparation of the alkoxides of *n*- and *iso*-pentyl alcohol by the following reactions :

$$Ta(O\cdot CH_2\cdot CH\cdot MeEt)_5 + 5CHMe_2\cdot CH_2\cdot CH_2\cdot OH \longrightarrow Ta(O\cdot CH_2\cdot CH_2\cdot CHMe_2)_5 + 5CHMeEt\cdot CH_2\cdot OH$$

$$Ta(O\cdot CH_2\cdot CH_2\cdot CHMe_2)_5 + 5n\text{-}C_5H_{11}\cdot OH \longrightarrow Ta(O\cdot C_5H_{11}\text{-}n)_5 + 5CHMe_2\cdot CH_2\cdot CH_2\cdot OH$$

The other tantalum alkoxides were obtained by reactions involving tantalum pentachloride and the appropriate alcohol in the presence of excess of ammonia :

$$TaCl_5 + 5ROH + 5NH_3 \longrightarrow Ta(OR)_5 + 5NH_4Cl$$

When secondary or tertiary alcohols were used an excess of pyridine was added to the pentachloride before the addition of the alcohol. This procedure obviated side reactions which occurred in the absence of pyridine.

*Isomeric Pentabutoxides of Tantalum.*—The normal physical appearance of the butoxides did not change regularly with the extent of chain branching of the butyl group. Thus the normal and secondary butoxides were pale yellow mobile liquids at room temperature but the *iso*butoxide (m. p. *ca.* 157°) and the tertiary butoxide (m. p. 110°) were solid. The high melting point of the *iso*butoxide made it necessary to determine boiling points of the butoxides under 5·5 mm. pressure for comparative purposes. These data and the molecular weights in benzene are shown in Table 1. They confirm the expected increase in volatility

Table 1.

| R in $Ta(OR)_5$ | B. p./5·5 mm. | $\Delta T_B$ | $M$ | Mol. complexity |
|---|---|---|---|---|
| $Me\cdot CH_2\cdot CH_2\cdot CH_2\cdot$ | 242° | — | 1102 [a] | 2·02 |
| $Me_2CH\cdot CH_2\cdot$ | 210 | 32° | 1094 [a] | 2·00 |
| $Me\cdot CH_2\cdot CHMe\cdot$ | 156 | 54 | 593 [a] | 1·06 |
| $Me_3C\cdot$ | 149·5 | 6 | 554 [b] | 1·01 |

[a] Ebullioscopic method. [b] Cryoscopic method.

with increased branching of the butyl group. However, the increment in boiling point ($\Delta T_B$) from one isomer to the next shows an abrupt change when passing from the *iso*-butoxide to the *sec.*-butoxide. Further, this effect is clearly reflected, at the same point, in the molecular complexities. Hence it appears that polymerisation, a consistent feature

* Part IV, *J.*, 1953, 2025.

[1] Bradley, Wardlaw, and Whitley, *J.*, 1955, 726.
[2] *Idem*, *J.*, 1956, 5.
[3] Bradley, Mehrotra, and Wardlaw, *J.*, 1952, 2027, 4204, 5020; Bradley, Mehrotra, Swanwick, and Wardlaw, *J.*, 1953, 2025; Bradley, Saad, and Wardlaw, *J.*, 1954, 3488.

of tantalum *n*-alkoxides, is prevented in the secondary and tertiary butoxides by the steric effect of branched butyl groups. The small difference in the boiling points ($\Delta T_B$ 6°) of the *sec.*- and *tert.*-butoxides is clearly a consequence of their monomeric nature which would cause a similarity in intermolecular forces. The higher melting point of the *tert.*-butoxide is especially interesting and suggests that this compound, by virtue of its more compact and nearly spherical molecules, possesses greater rotational freedom than the *sec.*-butoxide in the solid state. Earlier work on the Group IVA alkoxides established [3] that the two main factors which determined the volatility and molecular complexity of metal alkoxides were (i) the degree of branching and size of alkyl group and (ii) the atomic radius of the central atom. According to Sidgwick,[4] the atomic radii of tantalum and titanium are 1·34 and 1·36 Å respectively and therefore a comparison of tantalum penta-alkoxides with the corresponding tetra-alkoxides of titanium should reveal the part played by a new factor, *i.e.*, the number of groups attached to the central atom. The boiling points of titanium isomeric butoxides under 0·1 mm. pressure are [3]: *n* 142°; *iso* 106°; *sec.* 81°; *tert.* 50°. In this series it is noteworthy that the volatility increases steadily with increased branching of the butyl group from normal to tertiary in contrast to the abrupt change from *iso*- to *sec.*-butoxide for the tantalum derivatives. This shows the greater screening effect of five alkoxide groups in the tantalum compounds compared with four of the same alkoxides in the titanium compounds. Comparing monomeric compounds, it is not surprising to find that titanium tetra-*tert.*-butoxide $TiO_4C_{16}H_{36}$ (b. p. 96°/5·7 mm.), which has 57 atoms per molecule, has a lower boiling point than tantalum penta-*tert.*-butoxide $TaO_5C_{20}H_{45}$ (b. p. 149·5°/5·5 mm.) which has 71 atoms per molecule. On the other hand titanium tetra-*tert.*-pentyloxide, which has 69 atoms per molecule, has a boiling point (143°/5·0 mm.) quite close to that of tantalum penta-*tert.*-butoxide and the small difference could easily be due to the additional oxygen and hydrogen atoms in the tantalum compound. Thus the following centrosymmetrical molecules give rise to liquids of similar volatility: $Ti(O{\cdot}CMe_2Et)_4$; $Zr(O{\cdot}CMe_2Et)_4$; $Hf(O{\cdot}CMe_2Et)_4$; $Ta(O{\cdot}CMe_3)_5$. This behaviour is in accord with our theory that in these molecules the central metal atom is so effectively screened by its organic envelope that it can make only a minor contribution to the energy of intermolecular attraction in the liquid state. The major contribution must arise from interactions involving the peripheral carbon and hydrogen atoms, whilst the atomic weight or valency of the metal atom is relatively unimportant.

In view of the interesting behaviour of tantalum normal alkoxides in their own alcohols [2] the molecular weight of the *iso*butoxide was determined in boiling *iso*butyl alcohol (b. p. 107°). The molecular complexity found (1·47) was significantly higher than that (1·40) of the *n*-butoxide in *n*-butanol (b. p. 117°). If it is assumed that the donor tendencies of these alcohols are very similar, it appears that the lower complexity of the *n*-butoxide is caused by the higher boiling point of *n*-butanol, because any stereochemical effect due to chain branching in the *iso*butoxide or *iso*butyl alcohol would tend to lower the complexity of the *iso*butoxide. This supports our previous conclusion [2] that the molecular complexity of an alkoxide in its own alcohol is mainly determined by the boiling point and donor power of the alcohol.

TABLE 2.

| R in $Ta(OR)_5$ | Appearance | B. p./0·1 mm. | $\Delta T_B$ | *M* | Mol. complexity |
|---|---|---|---|---|---|
| $Me{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}$ | Liquid | 233° | — | 1239 | 2·01 |
| $Me_2CH{\cdot}CH_2{\cdot}CH_2{\cdot}$ ..... | ,, | 210 | 23° | 1223 | 1·98 |
| $MeEtCH{\cdot}CH_2{\cdot}$ ..... | Very viscous | 204 | 6 | 1216 | 1·97 |
| $Me_3C{\cdot}CH_2{\cdot}$ ..... | Solid (m. p. 90°) | 130 | 74 | 831 | 1·35 |
| $Et_2CH{\cdot}$ ..... | Very viscous | 153 | (−)23 | 626 | 1·02 |
| $MePr^nCH{\cdot}$ ..... | Liquid | 148 | 5 | 614 | 0·99 |
| $MePr^iCH{\cdot}$ ..... | Viscous | 137 | 11 | 625 | 1·02 |
| $Me_2EtC{\cdot}$ ..... | ,, | 139 | (−)2 | 619 | 1·00 |

*Isomeric Pentapentyloxides of Tantalum.*—The data on the pentyloxides are set out in Table 2. The molecular weights were determined ebullioscopically in benzene. Again

[4] Sidgwick, "Chemical Elements and their Compounds," Clarendon Press, Oxford, 1950, Vol. I, p. xxix.

it is evident that increased branching of the alkyl groups causes a decrease in molecular complexity and an increase in volatility. Considering first the primary pentyloxides, the behaviour of the *neo*pentyloxide is outstanding since it is the only primary derivative exhibiting a molecular complexity appreciably less than two and it is strikingly more volatile than any of the others. In fact, the *neo*pentyloxide is even more volatile than the monomeric secondary or tertiary pentyloxides, and its molecular complexity of 1·35 in boiling benzene seemed anomalously high. However, repeated ebullioscopic determinations of the molecular weight gave the same result whilst cryoscopic measurements in benzene gave a value of 1·39 for the complexity. This anomalous polymerisation of the *neo*pentyloxide is not confined to the tantalum derivative because earlier work [3] on the Group IVA alkoxides showed a similar but less pronounced effect in the pentyloxides of titanium and zirconium. Thus titanium tetra*neo*pentyloxide (m. p. 64°), with a molecular complexity of 1·3 in boiling benzene, was more volatile than the liquid monomeric secondary pentyloxides. In the case of zirconium the *neo*pentyloxide (m. p. 150—160°) had a complexity of 2·4 although it was close in volatility to the dimeric secondary pentyloxides. It is significant that the *neo*pentyloxides of titanium, tantalum, and zirconium are crystalline, with relatively high melting points compared with the corresponding secondary pentyloxides. Hence the anomalous complexities of the *neo*pentyloxides may not be caused by intermolecular forces between metal and oxygen but instead may be due to the factors which produce the high melting points of these compounds. In particular we believe that the high melting points arise from the compactness and nearly spherical nature of alkoxides containing the *neo*pentyl group (cf. the behaviour of secondary and tertiary butoxides of tantalum). A comparison of the primary pentyloxides of tantalum (Table 2) with the isomeric butoxides (Table 1) reveals the greater screening achieved by branching at the carbinol-carbon compared with similar branching at the β-carbon of the alkoxide group. Thus, for the butoxides an abrupt change in volatility and molecular complexity occurs between the *iso*butoxide and *sec.*-butoxide while in the primary pentyloxides a similar change occurs between the derivatives of *iso*pentyl and *neo*pentyl alcohol.

It is not surprising to find that all the secondary pentyloxides of tantalum are monomeric and consequently close in volatility to the monomeric tertiary pentyloxide. In fact the order of volatilities of the monomeric pentyloxides is: $Me_2EtC \sim MePr^iCH > MePr^sCH > Et_2CH$. We have already suggested that the monomeric penta-*tert.*-butoxide of tantalum should have a similar boiling point to the monomeric tetra(tertiary pentyloxides) of titanium, zirconium, and hafnium. For the same reason we should expect the boiling point of the tertiary pentyloxide of tantalum to be near to the boiling points of the monomeric tetrahexyloxides of titanium and zirconium. From previous work [3] the following boiling points under 0·1 mm. pressure have been calculated: $Ti(O{\cdot}CMeEt_2)_4$ 128°; $Zr(O{\cdot}CMeEt_2)_4$ 130°; $Ti(O{\cdot}CHEtPr^n)_4$ 134°; $Ti(O{\cdot}CHMeBu^n)_4$ 126°; $Ti(O{\cdot}CHMeBu^t)_4$ 127°; $Zr(O{\cdot}CHMeBu^t)_4$ 128°. It appears that the boiling points for monomeric hexyloxides of titanium and zirconium are *ca.* 130°/0·1 mm. and compare well with $Ta(O{\cdot}CHMePr^i)_5$ 137°/0·1 mm. and $Ta(O{\cdot}CMe_2Et)_5$ 139°/0·1 mm.

## Experimental

Details of the experimental technique and apparatus have already been described.[1,2] *tert.*-Butyl alcohol was dried by refluxing it over quick-lime, distilling, and refluxing the distillate over sodium. The other alcohols were fractionally distilled and the purest fractions were dried azeotropically with benzene. Pyridine ("AnalaR") was refluxed over potassium hydroxide (pellets), then distilled, and the distillate dried azeotropically with benzene.

*Tantalum Pentaisopentyloxide.*—Benzene (100 c.c.) and *iso*pentyl alcohol (30 c.c.) were added to tantalum penta-2-methylbutoxide (10 g.; preparation given below), and alcohol interchange was effected by fractional distillation. After evaporation of the residual solvent the new *alkoxide* was distilled (b. p. 210°/0·1 mm.) [Found: Ta, 29·3. $Ta(OC_5H_{11})_5$ requires Ta, 29·4%].

*Tantalum Penta*-n-*pentyloxide.*—Alcohol interchange was conducted on a mixture of tantalum penta*iso*pentyloxide (7 g.), benzene (100 c.c.), and *n*-pentyl alcohol (20 c.c.) in the foregoing manner. The new *alkoxide* distilled at 239°/0·2 mm. (Found: Ta, 29·3%).

*Preparation of Tantalum Alkoxides from the Pentachloride.*—Details of the preparation by the ammonia method in the absence of pyridine have been reported.[1] When pyridine was used the procedure was modified in order to complete the removal of chloride. The following preparation of tantalum penta-*tert.*-butoxide is typical: Pyridine (200 c.c.) and *tert.*-butyl alcohol (20 c.c.) were allowed to react with tantalum pentachloride (10 g.), and the solution was treated with ammonia in excess. After filtration and evaporation of solvent a white solid (Found: Cl, 0·39%) remained. This was dissolved in *tert.*-butyl alcohol (20 c.c.) and benzene (100 c.c.) and treated with ammonia. Removal of the finely divided ammonium chloride was effected with the aid of "Hyflo-supercel" and a sintered glass filter (porosity no. **4**). The filtrate was evaporated to dryness and gave a chloride-free wax (10 g.) [Found: Ta: 33·1; $Ta(OC_4H_9)_5$ requires Ta 33·1%]. A sample sublimed unchanged at 82°/0·05 mm. The remainder of the results are presented in Table 3.

TABLE 3.

| R | ROH (c.c.) | $TaCl_5$ (c.c.) | $C_5H_5N$ (c.c.) | $C_6H_6$ (c.c.) | $Ta(OR)_5$ Yield (g.) | B. p.°/mm. | Found : Ta (%) |
|---|---|---|---|---|---|---|---|
| Me·$CH_2$·$CH_2$·$CH_2$· | 20 | 10·0 | — | 200 | 14·0 | 217/0·15<br>242/5·5 | 33·16 |
| $Me_2CH$·$CH_2$· ..... | 20 | 10·0 | — | 200 | 13·5 | 155/0·1 (subl.)<br>210/5·5 | 33·2 |
| MeEt·CH· ..... | 40 | 10·0 | 100 | 150 | 10·0 | 123/0·02<br>156/5·5 | 33·0 |
| MeEtCH·$CH_2$· ... | 40 | 10·0 | — | 200 | 14·0 | 206/0·15 | 29·3 |
| $Me_3C$·$CH_2$· ..... | 15 | 10·0 | — | 150 | 15·0 | 136/0·2 | 29·3 |
| $Et_2CH$· ..... | 10 | 5·0 | 150 | — | 5·0 | 168/0·6 | 29·3 |
| $MePr^nCH$· ..... | 10 | 5·0 | 100 | — | 6·0 | 148/0·1 | 29·4 |
| $MePr^iCH$· ..... | 10 | 5·0 | 100 | — | 4·0 | 137/0·1 | 29·3 |
| $Me_2EtC$· ..... | 60 | 10·0 | 200 | 100 | 14·5 | 145/0·2 | 29·4 |

*Molecular Weights.*—The ebulliometric method has already been described [1,2] and the results are given in Table 4. They include an anomalously high value (1·46) for the complexity of tantalum tetra-*tert.*-butoxide, due to the appreciable volatility of this compound in boiling benzene as proved by the detection of tantalum in the distillate.

TABLE 4.

| R in $Ta(OR)_5$ | Range of *m* (g.) | Benzene (g.) | $\Delta T/m$ | *M* Found | *M* Calc. |
|---|---|---|---|---|---|
| Me·$CH_2$·$CH_2$·$CH_2$· ..... | 0·075—0·290 | 15·93 | 0·168° | 1102 | 546 |
| $Me_2CH$·$CH_2$· ..... | 0·047—0·298 | 15·50 | 0·174 | 1094 | " |
| MeEtCH· ..... | 0·080—0·320 | 15·59 | 0·329 | 575 | " |
| Me·$CH_2$·$CH_2$·$CH_2$·$CH_2$ ..... | 0·063—0·387 | 16·20 | 0·147 | 1239 | 616 |
| $Me_2CH$·$CH_2$·$CH_2$· ..... | 0·057—0·244 | 15·77 | 0·153 | 1223 | " |
| MeEtCH·$CH_2$ ..... | 0·055—0·278 | 16·18 | 0·150 | 1216 | " |
| $Me_3C$·$CH_2$· ..... | 0·042—0·250 | 15·92 | 0·223 | 831 | " |
| $Et_2CH$· ..... | 0·048—0·455 | 16·51 | 0·285 | 627 | " |
| $MePr^nCH$· ..... | 0·059—0·326 | 15·31 | 0·314 | 614 | " |
| $MePr^iCH$· ..... | 0·038—0·205 | 16·30 | 0·289 | 625 | " |
| $Me_2EtC$· ..... | 0·045—0·390 | 14·70 | 0·324 | 619 | " |

*Cryoscopic Determinations.*—Molecular weights of the *tert.*-butoxide and the *neo*pentyloxide were determined cryoscopically in benzene, in an apparatus designed to exclude moisture. The results are given in Table 5.

TABLE 5.

| R in $Ta(OR)_5$ | Benzene (g.) | Alkoxide: *m* (g.) | $\Delta T°$ | *M* Found | R in $Ta(OR)_5$ | Benzene (g.) | Alkoxide: *m* (g.) | $\Delta T°$ | *M* Found |
|---|---|---|---|---|---|---|---|---|---|
| $Me_3C$· ..... | 14·90 | 0·153 | 0·095 | 552 | $Me_3C$·$CH_2$· | 14·62 | 0·124 | 0·050 | 867 |
| | | 0·340 | 0·210 | 556 | | | 0·340 | 0·140 | 851 |
| | | 0·450 | 0·280 | 553 | | | 0·470 | 0·190 | 866 |
| | | | Calc.: | 546 | | | | Calc.: | 616 |

We thank Imperial Chemical Industries Limited for a grant for the purchase of the tantalum pentachloride.

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# 238. *The Kinetics of Oxidation by Nitrous and Nitric Acid. Part III.* Oxidation of Oxalic Acid by Nitrous Acid.*

By (Miss) P. A. Vamplew and K. Singer.

The velocity of the reduction of nitrous acid in aqueous solution containing oxalic acid and oxalate is of first order with respect to " analytical " nitrous acid and of approximately first order with respect to mono-oxalate ion ($HC_2O_4^-$). Between 35° and 55° the second-order constant equals $5{\cdot}05 \times 10^{11} \exp(-26{,}900/RT)$ l. mole$^{-1}$ sec.$^{-1}$.

It was hoped to measure the rate of oxidation of oxalic acid in media containing nitric and/or nitrous acid over a range of conditions similar to those under which the oxidation of formic acid had been studied.[1,2] This proved to be impossible because of the slowness of the reaction in the accessible range of concentrations of oxalic acid. Satisfactory velocities were only obtained when an appreciable fraction of the oxalic acid in the reaction mixture was ionised, *i.e.*, mainly between pH 1 and 2.

The reaction was followed by measuring the rate of disappearance of " analytical " nitrous acid † (initial concentration usually $4{\cdot}7 \times 10^{-3}$M) in media containing 0·3—0·7M-oxalic acid partly neutralised by lithium hydroxide and made up to an ionic strength approx. 0·9M by addition of lithium perchlorate. The disappearance of nitrous acid was also measured in blank experiments, *i.e.*, in media containing no oxalic acid but equalling the corresponding reaction mixtures in acidity, ionic strength, and initial nitrous acid concentration.

Table 1. Initial $(HNO_2) = 4{\cdot}7 \times 10^{-3}$M. Total ionic strength: approx. 0·9M.

| | | | | Calc. from $K' = 0{\cdot}05$ | | | Calc. from $K' = 0{\cdot}1$ | | |
|---|---|---|---|---|---|---|---|---|---|
| Total oxalate * (M) | LiOH (M) | $k' \times 10^3$ (min.$^{-1}$) | $k_b \times 10^3$ (min.$^{-1}$) | $[H^+]$ (M) | $[HOx^-]$ (M) | $k \times 10^3$ (l. mole$^{-1}$ min.$^{-1}$) | $[H^+]$ (M) | $[HOx^-]$ (M) | $k \times 10^3$ (l. mole$^{-1}$ min.$^{-1}$) |
| *At 35°.* | | | | | | | | | |
| 0·58 | 0·54 | 1·55 | 0·25 | 0·003 | 0·54 | 2·87 | 0·005 | 0·545 | 2·82 |
| 0·80 | 0·64 | 1·81 | 0·225 | 0·012 | 0·65 | 2·78 | 0·01 | 0·65 | 2·79 |
| 0·80 | 0·44 | 1·55 | 0·282 | 0·035 | 0·48 | 3·22 | 0·03 | 0·47 | 3·29 |
| 0·80 | 0·29 | 1·26 | 0·281 | 0·06 | 0·35 | 3·59 | 0·10 | 0·39 | 3·23 |
| 0·78 | 0·128 | 0·915 | 0·267 | 0·11 | 0·24 | 3·71 | 0·165 | 0·29 | 3·16 |
| *At 45°.* | | | | | | | | | |
| 0·58 | 0·54 | 5·15 | 0·75 | 0·003 | 0·54 | 9·5 | 0·005 | 0·545 | 9·4 |
| 0·80 | 0·68 | 7·19 | 0·74 | 0·008 | 0·69 | 10·4 | 0·015 | 0·70 | 10·3 |
| 0·58 | 0·32 | 4·40 | 0·51 | 0·03 | 0·35 | 12·6 | 0·05 | 0·37 | 11·8 |
| 0·80 | 0·41 | 5·47 | 0·58 | 0·04 | 0·45 | 12·1 | 0·06 | 0·47 | 11·5 |
| 0·80 | 0·29 | 4·92 | 0·39 | 0·06 | 0·35 | 14·0 | 0·10 | 0·39 | 12·6 |
| 0·80 | 0·10 | 3·54 | 0·42 | 0·13 | 0·23 | 15·4 | 0·17 | 0·27 | 13·1 |
| *At 55°.* | | | | | | | | | |
| 0·51 | 0·48 | 17·1 | 0·99 | 0·002 | 0·48 | 35·6 | 0·005 | 0·49 | 34·9 |
| 0·61 | 0·38 | 19·3 | 1·17 | 0·03 | 0·41 | 47·0 | 0·03 | 0·41 | 47·0 |
| 0·60 | 0·30 | 17·0 | 0·94 | 0·05 | 0·35 | 48·6 | 0·06 | 0·36 | 47·3 |
| 0·80 | 0·31 | 17·9 | 1·18 | 0·06 | 0·37 | 48·5 | 0·10 | 0·41 | 43·7 |
| 0·68 | 0·14 | 12·6 | 0·97 | 0·09 | 0·23 | 54·8 | 0·14 | 0·28 | 45·0 |
| 0·80 | 0·10 | 12·6 | 0·58 | 0·13 | 0·23 | 54·8 | 0·17 | 0·27 | 46·7 |
| 0·68 | 0·086 | 11·3 | 0·86 | 0·12 | 0·21 | 53·9 | 0·17 | 0·26 | 43·5 |

* Concn. of oxalic acid + concn. of oxalate ions.

(1) Reproducible linear plots of $\log(HNO_2)$ *versus* time were obtained for reaction mixtures and controls in all cases; the slope of the former ($k_{exp.}$) usually exceeded that of

* Part II, *J.*, 1954, 2610.

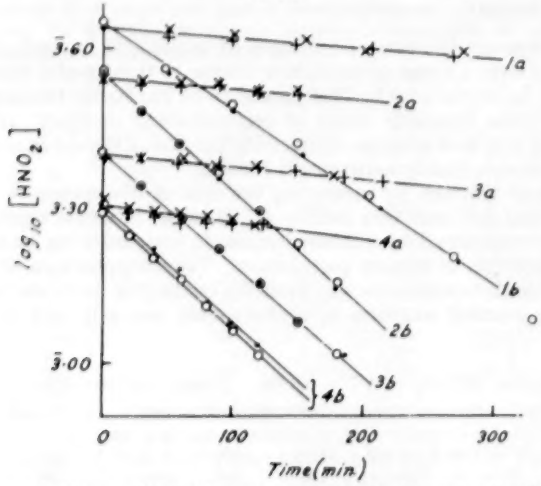
† The concentration of " analytical " nitrous acid, denoted by $(HNO_2)$, is the sum of the concentrations of all molecular entities which diazotise aromatic amines under the conditions of the colorimetric method used.

[1] Part I, Longstaff and Singer, *J.*, 1954, 2604.

[2] Part II, *idem*, *ibid.*, p. 2610.

the latter ($k_b$) by a factor of more than five. Some typical plots are shown in Fig. 1. Since the self-decomposition of nitrous acid by disproportionation ($3HNO_2 = HNO_3 + 2NO + H_2O$) has been shown to be of the fourth order with respect to "analytical" nitrous acid,[3] it seems probable that the nitrous acid in the blank experiments disappeared by evaporation. If one assumes that the rate of evaporation of nitrous acid in the reaction mixtures and in the blank experiments is approximately equal, the first-order constant for the reduction of nitrous acid ($k'$) is given by $k' = k_{exp.} - k_b$. Results obtained at 35°, 45°,

FIG. 1.




○ Runs at 45°. ● Duplicate runs at 45°. + × Corresponding blanks.

The ordinate scale of plots 2*a*—*b*, 3*a*—*b*, and 4*a*—*b* is displaced downwards by 0·1, 0·2, and 0·3 unit respectively.

| Run | Total oxalate (M) | LiOH (M) | Initial ($HNO_2$) (M × $10^2$) |
|---|---|---|---|
| 1 | 0·75 | 0·10 | 4·70 |
| 2 | 0·65 | 0·29 | 4·70 |
| 3 | 0·42 | 0·32 | 4·00 |
| 4 | 0·31 | 0·54 | 4·00 |

and 55° at initial ($HNO_2$) = $4{\cdot}7 \times 10^{-3}$M and ionic strength 0·9M (approx.) are shown in Table 1. In contrast to the reduction of nitrous acid by formic acid, $k'$ shows no trend with initial concentration of nitrous acid (Table 2).

TABLE 2.

No $LiClO_4$ added.

| | Total oxalate (M) | LiOH (M) | Initial ($HNO_2$) (M × $10^2$) | $k' \times 10^2$ ($min.^{-1}$) | Calc. from $K' = 0{\cdot}1$ [$H^+$] (M) | [$HOx^-$] (M) | $k \times 10^2$ ($min.^{-1}$) |
|---|---|---|---|---|---|---|---|
| At 35° | 0·66 | 0·64 | 4·0 | 1·54, 1·59 | 0·003 | 0·64 | 2·44 |
| | 0·66 | 0·64 | 2·0 | 1·52, 1·54 | 0·003 | 0·64 | 2·40 |
| | 0·66 | 0·64 | 1·0 | 1·51, 1·50 | 0·003 | 0·64 | 2·35 |
| At 45° | 0·52 | 0·48 | 4·0 | 5·94, 6·06 | 0·07 | 0·49 | 1·22 |
| | 0·52 | 0·48 | 2·0 | 5·33, 5·34 | 0·07 | 0·49 | 1·09 |
| | 0·52 | 0·48 | 1·0 | 5·79, 5·47 | 0·07 | 0·49 | 1·15 |

(2) Comparison between columns 3 and 4 of Table 1 suggests that $k'$ may be proportional to the concentration of mono-oxalate ion ($HC_2O_4^- = HOx^-$). The dissociation constant of oxalic acid and the activity coefficients of $H_2C_2O_4$, $H^+$, and $HC_2O_4^-$ at the required ionic

[3] Abel and Schmid, *Z. phys. Chem.*, 1928, **132**, 55; **134**, 279.

strength and temperatures are not known. When the concentration of mono-oxalate is calculated from $K' = K \cdot f_{H_2Ox}/f_{H^+} \cdot f_{HOx^-}[H^+][HC_2O_4^-]/[H_2C_2O_4] = 0{\cdot}05$, which is the value[4] of the thermodynamic dissociation constant of oxalic acid at zero ionic strength and 25°, the second-order constant $k = k'/[HC_2O_4^-]$ increases slowly with acidity. Approximately constant values for $k$ are obtained when the calculation is carried out for all temperatures with $K' = 0{\cdot}1$ (a value which in view of the high ionic strength is not unreasonable) (Table 1). The low values of $k$ at the lowest acidities can be ascribed to partial dissociation of nitrous acid (dissociation constant at 25° $= 5 \times 10^{-4}$ and quite possibly higher at the prevailing temperatures and ionic strengths). Thus, if $K_{HNO_2} = 5 \times 10^{-4}$ and $[H^+] = 7 \times 10^{-3}$, approximately 7% of the nitrous acid is dissociated.

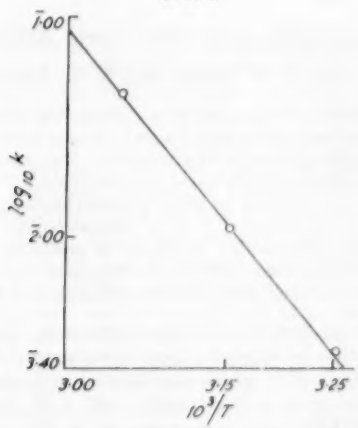
TABLE 3.

No $LiClO_4$ added.

| | Total oxalate (M) | LiOH (M) | $k' \times 10^3$ (min.$^{-1}$) | Calc. from $K' = 0{\cdot}1$ $[H^+]$ (M) | $[HOx^-]$ (M) | $k \times 10^3$ (min.$^{-1}$) |
|---|---|---|---|---|---|---|
| At 35° | 0·66 | 0·64 | 1·56 | 0·003 | 0·64 | 2·46 |
| | 0·58 | 0·53 | 1·55 | 0·004 | 0·53 | 2·92 |
| At 45° | 0·58 | 0·53 | 5·89 | 0·004 | 0·53 | 11·1 |
| | 0·58 | 0·32 | 4·63 | 0·03 | 0·35 | 13·2 |

(3) The omission of lithium perchlorate from the reaction mixture has no marked effect on $k$ (Tables 2 and 3).

FIG. 2.




(4) A plot of log $k$ (35°, 45°, and 55°) *versus* $1/T$ gave a straight line. For each temperature the mean was taken of the values of $k$ shown in Table 1, except the value obtained at the lowest acidity. It is found that $k = 5{\cdot}05 \times 10^{14} \exp(-26{,}900/RT)$ l. mole$^{-1}$ sec.$^{-1}$.

(5) Efforts to determine the stoicheiometry of the reaction by measuring the ratio of carbon dioxide formed to nitrous acid consumed were unsuccessful. Some results were however obtained showing that this ratio was not greater than 2 and not smaller than 1.

*Discussion.*—Since analytical nitrous acid in the reaction mixtures consists almost entirely of molecular nitrous acid (except at the lowest acidities employed), the first-order dependence on "analytical" nitrous acid excludes $N_2O_3$ or $NO_2$ as possible oxidising entities; oxidation by $N_2O_3$ would lead to the second order with respect to $(HNO_2)$, while oxidation by $NO_2$ would be compatible with a first-order reaction only in the initial stages of the reaction when $[NO_2] \propto (HNO_2)^2/[NO]$ and $[NO_2] = [NO]$, but not, as was observed, up to 70% conversion of nitrous acid. Oxidation of oxalate ion by $NO^+$ must be excluded

[4] Parton and Gibbons, *Trans. Faraday Soc.*, 1939, **35**, 542.

because of the slow (if any) variation of $k$ with acidity and with ionic strength. This leaves molecular nitrous acid as the probable oxidising agent.

### Experimental

"AnalaR" perchloric acid, sodium nitrite, oxalic acid, and B.D.H. lithium hydroxide were used without further purification.

Dissolved oxygen was removed as described in Part I.

Reaction mixtures were prepared by mixing solutions of known strengths of oxalic acid and of dilithium oxalate (prepared from oxalic acid and lithium hydroxide) in the required proportions, checking the excess of oxalic acid by alkalimetric titrations, adding calculated amounts of stock solutions of lithium perchlorate and of sodium nitrite, and diluting to 100 ml. with water.

It was found that a large volume of gas phase in contact with reaction mixture leads to appreciable loss of nitrous acid, *i.e.*, large and not always reproducible values for $k_b$. By using 100 ml. volumetric flasks as reaction vessels the volume of gas phase is kept sufficiently small—even after withdrawal of eight 2 ml. samples—to ensure small and reproducible values of $k_b$.

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## 239. α-*Methyleneadipic Acid and Some Related Compounds.*

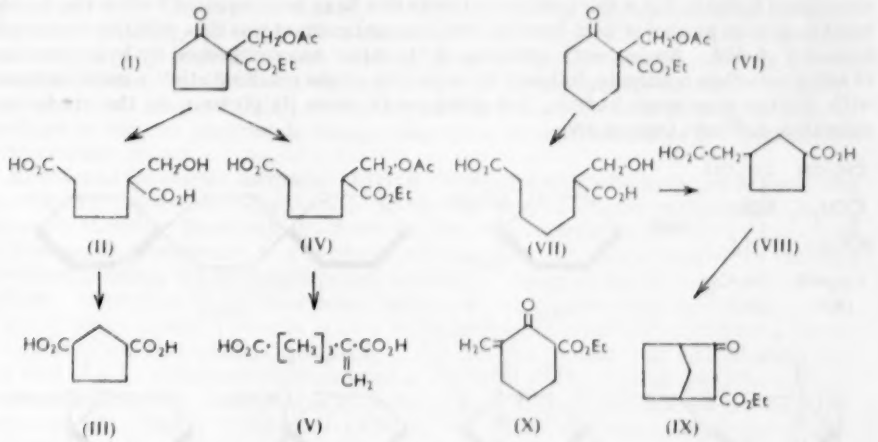
By L. N. Owen and A. G. Peto.

An alleged cyclodehydration reaction, which has been claimed to be applicable to the synthesis of *cyclo*pentane-1 : 3-dicarboxylic acid and *cyclo*pentane-1-acetic-3-carboxylic acid, is erroneous; the products are α-methyleneadipic and α-methylenepimelic acid, respectively. 2-Methylenehexane-1 : 6-diol, obtained by reduction of ethyl α-methyleneadipate, on ozonolysis gives 1 : 7 : 10 : 15-tetraoxa*dispiro*[5 : 2 : 5 : 2]hexadecane (XIII), a simple analogue of a diketose anhydride. Evidence is presented for the allocation of cyclic structures to an anhydro-2 : 4-dinitrophenylhydrazone and a 2 : 4-dinitrophenylosazone derived from 1 : 6-dihydroxyhexan-2-one.

As part of an investigation on hydroxyalkylation reactions, Gault and Daltroff [1,2] found that base-catalysed interaction of ethyl 2-oxo*cyclo*pentanecarboxylate and formaldehyde gave the 1-hydroxymethyl derivative, which was isolated as its acetate (I). After treatment with concentrated alkali this gave a dicarboxylic acid, $C_7H_{10}O_4$, which was stated to be saturated. It had m. p. 121·5°, gave a diamide, m. p. 224°, and on this evidence was identified as *cis-cyclo*pentane-1 : 3-dicarboxylic acid (III) (which has m. p. 121°; amide, m. p. *ca.* 228°), though no direct comparison with an authentic sample was made and all attempts to convert it into the well-known anhydride were unsuccessful. The formation of this acid was envisaged as proceeding by normal ring fission of the β-keto-ester (I), with hydrolysis, to give the intermediate hydroxy-dicarboxylic acid (II), which was then supposed to undergo cyclodehydration between the hydroxy-group and the α-methylene group adjacent to the remote carboxyl. In later papers [3,4] a similar reaction was carried out on the *cyclo*hexane analogue (VI) in the expectation that *cyclo*hexane-1 : 3-dicarboxylic acid would be obtained. The product, however, although a dicarboxylic acid of the correct molecular formula, had m. p. 85°, and was thus neither the *cis*- (m. p. 167°) nor the *trans*-form (m. p. 150°) of the *cyclo*hexane acid. Consequently it was assumed that on

[1] Gault and Daltroff, *Compt. rend.*, 1939, **209**, 997; *Chimie et Industrie*, 1941, **45 bis**, **122**.
[2] Daltroff, *Ann. Chim.* (*France*), 1940, **14**, 207.
[3] Gault and Hiong, *Compt. rend.*, 1941, **213**, 353.
[4] Hiong, *Ann. Chim.* (*France*), 1942, **17**, 269.

this occasion the elimination of water from an intermediate (VII) had involved a methylene group β to the remote carboxyl, to give *cyclo*pentane-1-acetic-3-carboxylic acid (VIII); the discrepancy in melting point from that (137°) recorded by Hintikka and Komppa [5] for this compound was ascribed to stereoisomerism. Hiong's attempt to convert it by



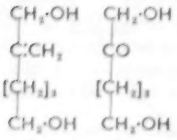

cyclisation into norcamphor was unsuccessful, though it underwent the Dieckmann reaction to give a product formulated as the norcamphorcarboxylic ester (IX), from which a corresponding hydroxy-ester and hydroxy-acid were prepared.

The simplicity of Daltroff's synthesis of *cyclo*pentane-1 : 3-dicarboxylic acid, if genuine, renders it very attractive, and we therefore repeated the preparation as described by her.[2] Recrystallisation of the acid obtained, however, raised the melting point to 128°; furthermore, it was unsaturated, absorbed one mol. of hydrogen over a palladium catalyst, and gave formaldehyde on ozonolysis. The product is therefore not the *cyclo*pentane acid, but α-methyleneadipic acid (V), which has recently been synthesised from acetylenic intermediates by Jones, Whitham, and Whiting.[6] This structure had been rejected by Daltroff [2] on the grounds that addition of bromine water to an *alcoholic* solution of the acid resulted in no decolorisation; we have confirmed this misleading observation, but in *aqueous* solution decolorisation is instantaneous. Daltroff's [2] suggested route for a synthesis of camphoric acid, based on a modification of the supposed cyclodehydration reaction, is of course invalidated. The formation of α-methyleneadipic acid from the acetoxymethyl compound (I) provides a new example of the conversion [7] of a β-acyloxy-ester, by base-catalysed elimination, into an αβ-unsaturated acid, and probably proceeds through the intermediate (IV).

The above observations rendered it highly probable that Hiong's conclusions,[4] based on an even more unlikely mode of dehydration, were also erroneous, and the alkaline fission of the keto-ester (VI) was therefore investigated. The product was again an *unsaturated* acid, identical with the α-methylenepimelic acid synthesised by Jones *et al.*[6] Hiong's failure to convert it into norcamphor is thus understandable and his product of Dieckmann cyclisation must be reformulated as (X) (or the isomer with an endocyclic double bond) with corresponding modifications to the structures of the compounds derived from this. It is worth emphasising that the French workers were led into a sequence of errors entirely as a result of an unreliable method for the detection of unsaturation by a bromine–water test.

[5] Hintikka and Komppa, *Ann. Acad. Sci. Fennicae*, 1918, **10**, *A*, 1.
[6] Jones, Whitham, and Whiting, *J.*, 1954, 1865.
[7] Linstead, Owen, and Webb, *J.*, 1953, 1211.

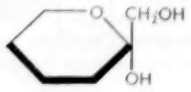
The ready availability of α-methyleneadipic acid by Daltroff's procedure led us to attempt the preparation of the corresponding glycol. Reduction of either the methyl or the ethyl ester of the acid with lithium aluminium hydride gave a product which although mainly 2-methylenehexane-1 : 6-diol (XI) was shown by quantitative hydrogenation to contain some saturated impurity. Ethylenic linkages are normally unaffected by lithium aluminium hydride, but a few instances of reduction have been reported [8] when the double bond is αβ to an alcohol or keto-function, and the contaminant was thus probably 2-methylhexane-1 : 6-diol. An authentic specimen of the latter was synthesised by hydrogenation of ethyl α-methyleneadipate, followed by reduction of the resulting ethyl α-methyladipate with lithium aluminium hydride, but attempts to prove its presence in the crude unsaturated diol were inconclusive.



(XI)

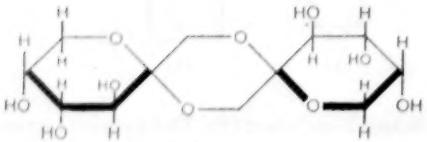


(XII)

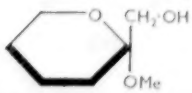


(XII*a*)

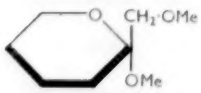
(XIII)



(XIV)



(XV)



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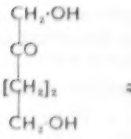

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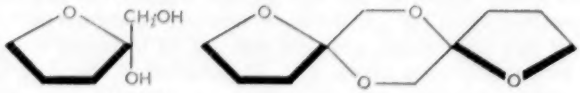
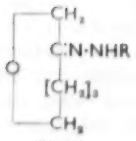
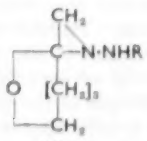


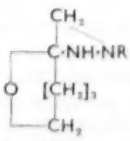
(XVIII)



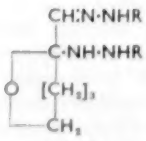
(XIX)



(XX)



(XXI)



(XXII)

(R = 2 : 4-dinitrophenyl)

Ozonolysis of the unsaturated diol would be expected to give 1 : 6-dihydroxyhexan-2-one (XII), a compound of interest as a simple analogue of the ketohexoses, particularly as it should exist in the pyranose form (XII*a*). When the ozonolysis was carried out, formaldehyde was produced, and after decomposition of the ozonide with zinc and acetic acid a crystalline solid $C_{12}H_{20}O_4$ (analysis and molecular weight) was obtained; this formula corresponds to two mols. of the expected ketol (XII) less two mols. of water. It was neutral, slowly reduced boiling Fehling's solution, and gave no reaction with 2 : 4-dinitrophenylhydrazine in cold aqueous sulphuric acid, though when heated with the latter reagent it gave a 2 : 4-dinitrophenylosazone. The substance is therefore 1 : 7 : 10 : 15-tetraoxa*dispiro*[5 : 2 : 5 : 2]hexadecane (XIII), derived by intermolecular dehydration from

[8] Brown, *Organic Reactions*, 1951, **6**, 481; Jenny and Grob, *Helv. Chim. Acta*, 1953, **36**, 1936.

the pyranose form (XIIa) of the ketol. Because of the presence of the two *spiro*-systems it can exist in a racemic and in a *meso*-form, but on the present evidence an allocation of configuration is not possible (the structure shown is the *meso*-form). The compound is thus the simplest analogue of diheterolevulosan-I (XIV), one of the products obtained [9] by dehydration of fructose; the corresponding bimolecular dianhydride of sorbose has recently been prepared.[10] The furanose analogue of (XIII), 1 : 6 : 9 : 13-tetraoxa*dispiro*[4 : 2 : 4 : 2]-tetradecane (XVIII), which has been obtained [11] from tetrahydro-2 : 3-dihydroxypyran, and also [12] as a by-product from the hydrogenation of furfuryl alcohol, is reported to be readily hydrolysed by dilute aqueous acid to give 1 : 5-dihydroxypentan-2-one (XVII), but, in contrast, fission of (XIII) with dilute acid does not occur readily, and the relative stabilities of the two compounds thus parallel those of the furanosides and pyranosides of the common sugars.

Reaction of the *dispiro*-compound (XIII) with boiling methanolic hydrogen chloride was expected to give the "methyl glycoside" (XV), but the product gave analyses for $C_8H_{16}O_3$ and must therefore be tetrahydro-2-methoxy-2-methoxymethylpyran (XVI); possibly the primary hydroxy-group in a product (XV) may be sufficiently reactive to undergo etherification during the prolonged period necessary to bring all the starting material into reaction. Hydrolysis of the dimethoxy-compound with aqueous acid gave a non-homogeneous product (probably because of some accompanying elimination) which gave a yellow 2 : 4-dinitrophenylhydrazone, $C_{12}H_{14}O_5N_4$. This formula contains one mol. of water less than that of a 2 : 4-dinitrophenylhydrazone of the ketol (XII); furthermore, the infrared spectrum indicated the absence of a hydroxyl group. Possible formulæ for the anhydro-compound are (XIX), (XX), and (XXI). The ultraviolet light absorption, though similar to that of a simple 2 : 4-dinitrophenylhydrazone, does not necessarily favour (XIX), as the other two structures represent unknown systems. The derivative, with excess of the reagent, gave the normal 2 : 4-dinitrophenylosazone, described above. This, also, showed no infrared absorption corresponding to the presence of a hydroxyl group, which suggests that it exists as the cyclic structure (XXII). There has been controversy over the structures of the sugar osazones,[13] and it is still uncertain whether the sugar chain is in the cyclic or acyclic form. Simple compounds of the type (XII), which are free from the complications brought about by the presence of the larger number of hydroxyl groups in true sugars, offer obvious possibilities for the study of the osazone problem, and it is intended to continue this aspect of the work.

## Experimental

Ethyl 1-acetoxymethyl-2-oxo*cyclo*pentanecarboxylate, b. p. 122—123°/0·4 mm., $n_D^{17}$ 1·4542, was obtained in an overall yield of 68% from ethyl 2-oxo*cyclo*pentanecarboxylate by the method of Daltroff,[2] who recorded b. p. 160—162·5°/17 mm. and 53% yield.

*α-Methyleneadipic Acid.*—The acetoxy-ester (117 g.) was cautiously added to sodium hydroxide (90 g.) in 50% aqueous ethanol (900 c.c.). When the exothermic reaction had subsided the solution was boiled under reflux for 2 hr. and then concentrated under reduced pressure to small bulk and acidified by the addition of 17% hydrochloric acid (450 c.c.). The mixture was cooled to 0°, and the precipitated solid was collected, dried in a vacuum, and extracted with ether (Soxhlet). Evaporation of the ethereal extract gave α-methyleneadipic acid (76 g., 88%), m. p. 124°, raised to 128° on recrystallisation from water, undepressed on admixture with an authentic specimen (Found: C, 53·3; H, 6·4%; equiv., 79·8; hydrogenation no., 159. Calc. for $C_7H_{10}O_4$: C, 53·2; H, 6·4%; equiv., 79·1; *M*, 158). Light absorption in EtOH: no max. >2100 Å. Ozonolysis of the acid in acetic acid gave formaldehyde (dimedone derivative, m. p. and mixed m. p. 188°).

Reaction of the acid with boiling methanolic hydrogen chloride gave the methyl ester (96%), b. p. 117°/8 mm., $n_D^{21}$ 1·4469. Daltroff,[2] for "methyl *cyclo*pentane-1 : 3-dicarboxylate," recorded b. p. 126—130°/17 mm.

[9] Schlubach and Behre, *Annalen*, 1933, **508**, 16.
[10] Wolfrom and Hilton, *J. Amer. Chem. Soc.*, 1952, **74**, 5334.
[11] Hurd and Edwards, *J. Org. Chem.*, 1949, **14**, 680.
[12] Swadesh and Dunlop, *ibid.*, p. 692.
[13] Percival, *Adv. Carbohydrate Chem.*, 1948, **3**, 23; Barry, McCormick, and Mitchell, *J.*, 1955, 222.

The acid (23·7 g.), ethanol (80 c.c.), carbon tetrachloride (250 c.c.), and toluene-*p*-sulphonic acid (1·5 g.), boiled together with azeotropic removal of the water formed, gave the *ethyl ester* (28·1 g.), b. p. 94°/0·2 mm., $n_D^{17}$ 1·4436 (Found: C, 61·8; H, 8·55. $C_{11}H_{18}O_4$ requires C, 61·6; H, 8·5%).

*α-Methylenepimelic Acid.*—When carried out by Hiong's procedure,[4] reaction of ethyl 1-acetoxymethyl-2-oxocyclopentanecarboxylate with 40% aqueous sodium hydroxide gave a product which, after recrystallisation from benzene, furnished α-methylenepimelic acid, m. p. and mixed m. p. 87—88°. An aqueous solution instantly decolorised bromine water, but decolorisation was very slow when bromine water was added to an alcoholic solution of the acid.

*Ethyl α-Methyladipate.*—A solution of ethyl α-methyleneadipate (6·6 g.) in ethanol (90 c.c.) was shaken with 10% palladium–charcoal (1 g.) and hydrogen until no further absorption occurred. Distillation of the product gave ethyl α-methyladipate (6·1 g.), b. p. 128—129°/14 mm., $n_D^{19}$ 1·4270. Dieckmann [14] gives b. p. 127—129°/13 mm.

*2-Methylhexane-1 : 6-diol.*—A solution of ethyl α-methyladipate (6·3 g.) in dry ether (23 c.c.) was added to a stirred solution of lithium aluminium hydride (1·9 g.) in dry ether (15 c.c.). The mixture was boiled under reflux for 3 hr., then cooled, cautiously treated with water, and acidified with dilute sulphuric acid. The ethereal layer was removed, and the aqueous portion was continuously extracted with ether for 24 hr. The combined ethereal extracts were dried ($Na_2SO_4$) and evaporated to give an oil which still contained some unchanged ester (saponification test). It was therefore boiled under reflux with N-sodium hydroxide in 50% ethanol (30 c.c.) for 4 hr. The ethanol was then removed by evaporation, and extraction of the alkaline solution with ether furnished *2-methylhexane-1 : 6-diol* (1·4 g.), b. p. 105—107°/0·1 mm., $n_D^{21}$ 1·4556 (Found: C, 63·8; H, 12·3. $C_7H_{16}O_2$ requires C, 63·6; H, 12·2%).

Treatment of the diol with a slight excess of naphthyl *iso*cyanate for 3 hr. at 100° in a sealed tube gave the *di-α-naphthylurethane*, m. p. 152° (from aqueous ethanol) (Found: C, 74·0; H, 6·5; N, 6·2. $C_{29}H_{30}O_4N_2$ requires C, 74·0; H, 6·4; N, 5·95%).

*2-Methylenehexane-1 : 6-diol.*—A solution of methyl α-methyleneadipate (4 g.) in dry ether (50 c.c.) was added during 20 min. to a stirred solution of lithium aluminium hydride (1·2 g.) in dry ether (80 c.c.) at 0°. Stirring was maintained for a further hour, and the product was isolated as described above. Distillation gave (i) 0·8 g., b. p. 60—90°/0·05 mm., $n_D^{19}$ 1·4499—1·4575, containing some unchanged ester, and (ii) 1·2 g., b. p. 90—95°/0·05 mm., $n_D^{19}$ 1·4732—1·4742. Redistillation of (ii) gave *2-methylenehexane-1 : 6-diol*, b. p. 122°/0·4 mm., $n_D^{18}$ 1·4749 (Found: C, 64·4; H, 10·8. $C_7H_{14}O_2$ requires C, 64·6; H, 10·8%). The *di-α-naphthylurethane* had m. p. 115—116° [from light petroleum (b. p. 60—80°)] (Found: C, 74·6; H, 6·3; N, 6·2. $C_{29}H_{26}O_4N_2$ requires C, 74·3; H, 6·0; N, 6·0%).

Higher yields of the diol (50—60%) were obtained by performing the reaction for the same time in boiling ether, but the products were still not homogeneous. Quantitative hydrogenation of a fraction, b. p. 103°/0·3 mm., $n_D^{17}$ 1·4760, in ethanol over a platinum catalyst, resulted in the absorption of only 70% of the theoretical amount of hydrogen.

*Ozonolysis of 2-Methylenehexane-1 : 6-diol.*—Ozonised oxygen was passed through a solution of the diol (3·9 g.) in dry ethyl acetate (90 c.c.) at 0°, and the issuing gases were led through a small wash-bottle containing water. When no more ozone was absorbed, the ethyl acetate was removed under reduced pressure, and the oily residue was heated on the steam-bath with water (20 c.c.), acetic acid (80 c.c.), and zinc dust (10 g.) for 30 min. A portion of the filtered solution gave with dimedone a precipitate of the formaldehyde derivative, and a further quantity was isolated by similar treatment of the wash-water from the ozonisation. The yield of the derivative, m. p. and mixed m. p. 187°, corresponded to a yield of 20% of formaldehyde. Another portion of the filtrate, on treatment with aqueous 2 : 4-dinitrophenylhydrazine sulphate, gave formaldehyde 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 161—162°, in quantity corresponding to a 24% yield. Pure compounds containing the $\cdot CH_2$ group usually give *ca.* 30% under these conditions of ozonolysis.

The residual filtrate was evaporated under reduced pressure to dryness and the residue was extracted with ether (Soxhlet) until the remaining zinc salts gave no ketonic reaction. Evaporation of the extract gave an oil, which was triturated with cold petroleum (b. p. 40—60°) (2 × 15 c.c., and 3 × 10 c.c.); the soluble material (0·3 g.), recovered from the petroleum solution, solidified, and on recrystallisation from methanol gave square plates or cubes of *1 : 7 : 10 : 15-tetraoxadispiro[5 : 2 : 5 : 2]hexadecane*, m. p. 161° [Found: C, 63·0; H, 8·9%; *M* (Rast),

[14] Dieckmann, *Annalen*, 1901, **317**, 69.

237. $C_{12}H_{20}O_4$ requires C, 63·1; H, 8·8%; *M*, 228], only sparingly soluble in water, neutral, and non-reducing to aqueous permanganate. It reduced Fehling's solution on prolonged boiling.

In another experiment, ozonolysis of the diol (8 g.) was followed by decomposition of the ozonide by hydrogenation of the ethyl acetate solution over palladium–charcoal. Evaporation of the filtered solution then gave a semi-solid residue, from which the *dispiro*-compound (1·9 g.) was isolated by trituration with water (4 × 2 c.c.) in the centrifuge, the residual solid then being recrystallised from methanol.

Further small quantities of the solid were obtained by treatment of the oily residues with boiling 2N-hydrochloric acid for 45 min., followed by evaporation, and extraction of the residue with light petroleum as before.

*Fission of* 1 : 7 : 10 : 15-*Tetraoxa*dispiro[5 : 2 : 5 : 2]*hexadecane.*—(i) The compound (36 mg.) was heated on the steam-bath with alcoholic 2 : 4-dinitrophenylhydrazine sulphate (24 c.c., containing 0·4 g. of base and 4·5 c.c. of sulphuric acid). Precipitation of a solid began after a few minutes, and after 1 hr. it was collected and washed with dilute sulphuric acid (yield, 141 mg., 84%). Recrystallisation from pyridine gave the red *6-hydroxy-2-oxohexanal di-2 : 4-dinitrophenylhydrazone*, m. p. 224—226° (Found: C, 44·2; H, 3·8; N, 22·8. $C_{18}H_{18}O_9N_8$ requires C, 44·1; H, 3·7; N, 22·85%). With aqueous sodium hydroxide and ethanol it gave the deep violet colour characteristic [15] of 2 : 4-dinitrophenylosazones. Light absorption max. in $CHCl_3$: *ca.* 3950 and 4380 Å (ε 35,300) (broad bands). Infrared absorption max. in paraffin mull: 3270 and 3160 cm.$^{-1}$ (NH); no indication of OH.

(ii) A solution of the compound (1·5 g.) in 3% methanolic hydrogen chloride (10 c.c.) was boiled under reflux for 17 hr., and then neutralised with silver carbonate. Evaporation of solvent from the filtered solution and distillation of the residue gave *tetrahydro-2-methoxy-2-methoxymethylpyran* (1·7 g.), b. p. 110°/50 mm., $n_D^{20}$ 1·4382 (Found: C, 60·0; H, 10·1; O, 29·95. $C_8H_{16}O_3$ requires C, 60·0; H, 10·0; O, 30·0%). Treatment of a portion with 2N-hydrochloric acid for 1 hr. on the steam-bath, followed by addition of aqueous 2 : 4-dinitrophenylhydrazine sulphate to the cooled solution, gave a sticky yellow precipitate. This was occasionally triturated during 2 hr., and was then collected, washed, and recrystallised from dioxan to give a yellow *compound*, m. p. 164° [Found: C, 48·8; H, 5·0; N, 19·3%; *M* (Rast), 299. $C_{12}H_{14}O_8N_4$ requires C, 49·0; H, 4·8; N, 19·0%; *M*, 294]. Light absorption max.: in EtOH, 3550 (ε 25,000); in $CHCl_3$, 3600 Å (ε 24,000). Infrared absorption max. in $CCl_4$: 3197 and 3098 cm.$^{-1}$ (NH); OH bands were absent.

The filtrate from the preparation of the derivative was treated with a further quantity of the reagent and heated on the steam-bath for 2 hr. to give a flocculent precipitate, which on recrystallisation from pyridine furnished the red 2 : 4-dinitrophenylosazone, m. p. 223—225°, identical with that described above. The same osazone was also obtained by heating the yellow derivative, m. p. 164°, with an excess of the reagent.

This work was carried out during the tenure (by A. G. P.) of a University of London Postgraduate Studentship. We are grateful to Professor E. R. H. Jones, F.R.S., for authentic specimens of the unsaturated acids. Microanalyses were by Mr. F. H. Oliver and his staff (Organic Microanalytical Laboratory), and the ultraviolet and infrared absorption spectra were determined by Mrs. A. I. Boston and Mr. R. L. Erskine, B.Sc., A.R.C.S., respectively (Organic Spectrographic Laboratory).

Department of Chemistry, Imperial College of Science and Technology,
South Kensington, London, S.W.7. [*Received, September 7th*, 1955.]

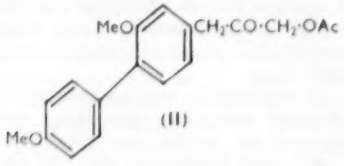
[15] Strain, *J. Amer. Chem. Soc.*, 1935, **57**, 758.

## 240. *Analogues of Cortical Hormones.*

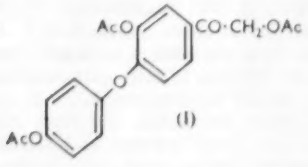
By Edward R. Clark and J. G. B. Howes.

2-3′-Acetoxyacetonyl*cyclo*pentanone (V) and 2-3′-acetoxyacetonyl-5-*p*-methoxyphenyl*cyclo*pentanone (IV; R = H) have been synthesized as analogues of 11-dehydrocorticosterone.

Walker [1] and Logemann [2] have synthesized simple analogues (I, II) of cortical hormones possessing phenolic groups in positions comparable with the 11-oxygen functions in certain of the natural hormones. Their compounds were physiologically inactive whereas the related structure (III),[3] not possessing this potential phenolic group, has proved effective for prolongation of the life of adrenalectomised animals. The 11-oxygen function is, however, essential if the hormone is to control protein and carbohydrate metabolism. It is not, of course, phenolic. The synthesis of compounds in which the analogous group is not phenolic might therefore be of interest.

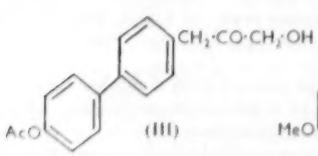



(II)

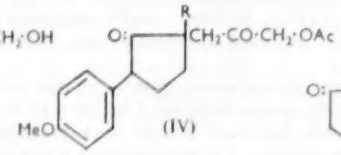



(I)

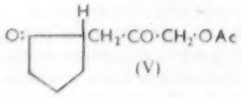
The *cyclo*pentanone (IV) was chosen for initial investigation, in preference to a *cyclo*hexane structure, since the introduction of the side chain would be facilitated. Synthesis of the single-ring analogue (V) was undertaken to gain experience of the steps involved.




(III)




(IV)




(V)

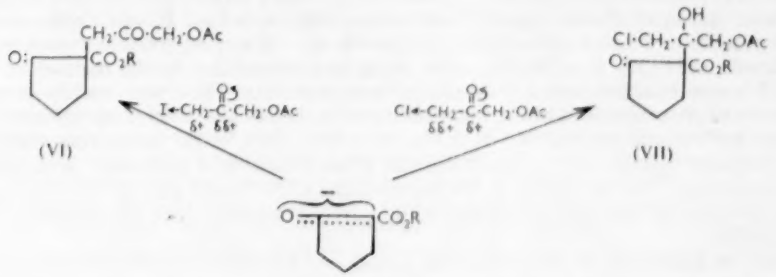
Reaction between α-halogeno-ketones and β-keto-esters does not always yield the *C*-subsitution product,[4] *O*-alkylation and aldol condensation being offered as explanations for the formation of alternative products. 1-Acetoxy-3-chloroacetone [5] (an improved method of isolation, including that of the co-formed 1 : 3-diacetoxyacetone, not isolated by the original workers, is described) reacted rapidly with the sodio-derivative of ethyl 2-oxo-*cyclo*pentanecarboxylate to yield only 9% (best) of the desired compound (VI; R = Et) together with a considerable amount of a non-distillable oil. 1-Acetoxy-3-iodoacetone (an unstable, non-distillable oil), on the other hand, reacted readily at 0° to give ethyl 1-3′-acetoxyacetonyl-2-oxo*cyclo*pentanecarboxylate (VI; R = Et) as the major product (72%), there being only a small amount of the by-product.

There is no reason to believe that the use of the chloride in place of the iodide will enhance the likelihood of *O*-substitution at the expense of *C*-substitution, both of which would occur more readily with the iodide. Here, therefore, the alternative product is due to aldol condensation to give the alcohol (VII; R = Et). Some support for this conclusion is found in the failure of 1-chloro-2 : 2-diethoxypropane [6] to react under similar conditions.

[1] Walker, *J.*, 1942, **347**.
[2] Logemann, *Z. physiol. Chem.*, 1952, **290**, 61.
[3] Linnell, Mathieson, and Williams, *Nature*, 1951, **167**, 237.
[4] Feist, *Ber.*, 1902, **35**, 1545; Archer and Pratt, *J. Amer. Chem. Soc.*, 1944, **66**, 1656; Dunlop and Hurd, *J. Org. Chem.*, 1950, **15**, 1160.
[5] Hess and Fink, *Ber.*, 1915, **48**, 1986.
[6] Ewlampiew, *Ber.*, 1929, **62**, 2386.

Ketonic hydrolysis of the diketo-ester (VI; R = Et), followed by reacetylation of the product, gave the desired analogue (V). Better yields were obtained by the acid-catalysed decomposition of the corresponding *tert.*-butyl ester (VI; R = $Bu^t$) under anhydrous conditions.

Dieckmann cyclisation of di-*tert.*-butyl adipate occurred readily, to give the cyclic β-keto-ester in good yield (80%) if an excess of condensing agent was used. The 3′-acetoxyacetonyl derivative (VI; R = $Bu^t$) was thermally unstable at 15 mm., 2-3′-acetoxyacetonyl*cyclo*pentanone (V) being formed. Better yields were obtained when the decomposition was catalysed with toluene-*p*-sulphonic acid.



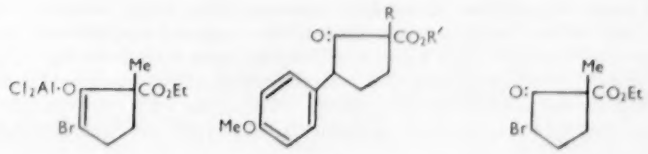
(VI) (VII)

The extension of this method to the synthesis of the two-ring analogue (IV; R = H) necessitated the synthesis of α-*p*-methoxyphenyladipic acid.

Reaction of γ-iodobutyronitrile with the sodio-derivative of *p*-methoxyphenylacetonitrile or *p*-methoxyphenylmalonate, followed by hydrolysis, failed to yield appreciable quantities of the desired acid (cf. Newman and Closson [7] and Case [8] who obtained α-methyl-α-phenyl- and α-phenyl-adipic acids respectively by analogous methods).

Ethyl γ-iodobutyrate with the sodiomalonate gave, however, an oil (70%) which on hydrolysis yielded the desired adipic acid (73%) together with some *p*-methoxyphenylacetic and *p*-methoxyphenylmalonic acid. These can only arise from the presence of the isomeric keto-ester (IX) together with the expected triester (VIII) in the product of condensation.

(VIII) (IX)



(XII) (X) (XI)

Esterification of the derived adipate, followed by Dieckmann cyclisation and methylation, yielded ethyl 3-*p*-methoxyphenyl-1-methyl-2-oxo*cyclo*pentanecarboxylate (X; R = Me, R′ = Et). The tedious nature of this method of preparation led us to examine other possible routes.

[7] Newman and Closson, *J. Amer. Chem. Soc.*, 1944, **66**, 1553.
[8] Case, *ibid.*, 1933, **55**, 2927.

Berlande [9] obtained α-phenyladipic acid by oxidation of 3-phenyl*cyclo*hexene. Oxidation of the product of reaction between *p*-methoxyphenylmagnesium bromide and 3-bromo*cyclo*hexene gave, however, a mixture of the required adipic acid and δ-anisoylvaleric acid. The ultraviolet absorption spectrum of the intermediate *cyclo*hexene indicated the presence of some of the conjugated isomer, thus accounting for the formation of the keto-acid. The experiments were abandoned because of the low yield of acids obtained and the failure to isolate a pure sample of 3-*p*-methoxyphenyl*cyclo*hexene (*i.e.*, one not possessing an absorption peak at 249 mμ).

Neutral permanganate oxidation of 2-*p*-methoxyphenyl*cyclo*hexanone gave δ-anisoylvaleric acid as the sole product.

Mason and Terry [10] and Richard [11] have successfully carried out Friedel-Crafts reactions between α-chloro-ketones and aromatic hydrocarbons. It seemed possible, therefore, that the dicyclic ester (X; R = Me, R′ = Et) might be obtained in a similar manner by using ethyl 3-bromo-1-methyl-2-oxo*cyclo*pentanecarboxylate (XI). This was readily produced by means of *N*-bromosuccinimide, but reaction with anisole and aluminium chloride failed to yield any dicyclic product (X; R = Me, R′ = Et): 64% of the bromo-compound (XI) was recovered together with a small amount of an unsaturated substance, presumably a *cyclo*pentenone. The formation of the *cyclo*pentene (XII) would explain the inert nature of the bromine and the vigorous evolution of hydrogen chloride which was observed during the reaction.

Since we attempted the above reaction Zaheer and his co-workers [12] have demonstrated the complex nature of the products which may be obtained by using aliphatic α-halogeno-ketones in reactions with aryl ethers and aluminium chloride.

Di-*tert.*-butyl α-*p*-methoxyphenyladipate, prepared *via* the di-acid chloride, was difficult to isolate in quantity since it could only be distilled in a short-path still. The product of Dieckmann cyclisation was even more unstable and could not be distilled even at $1 \times 10^{-5}$ mm. A portion of the product, presumably the required cyclic keto-ester (X; R = H, R′ = Bu$^t$) (colour with neutral ferric chloride), was separated from unchanged adipate by extraction with alkali, but decomposition soon occurred on storage.

The isolation of this unstable substance was avoided by adding 1-acetoxy-3-iodoacetone to the crude sodio-derivative obtained on cyclising di-*tert.*-butyl α-*p*-methoxyphenyladipate. Catalytic decarboxylation of the product yielded a small amount of the desired material (IV; R = H). No crystalline carbonyl derivative could be obtained but infrared spectroscopy showed strong absorption peaks at 5·69, 5·79, and 5·86 μ, corresponding with cyclic, acetate, and aliphatic carbonyl groups. This resembles the behaviour of dehydrocorticosterone acetate except that the alicyclic carbonyl band has been displaced to a shorter wavelength with diminution of ring size.

Disubstituted β-keto-esters are very sensitive to metal alkoxides. The use of the crude sodio-derivative in the above reaction meant that such a compound (IV; R = $CO_2Bu^t$) was being exposed to sodium *tert.*-butoxide. Since, however, this stage of the reaction was completed at room temperature, alcoholysis was considered to be unlikely. In order to confirm this, and before the above experiments were carried out, diethyl α-*p*-methoxyphenyladipate was cyclised and 1-acetoxy-3-iodoacetone added to the crude sodium derivative. Ethyl 1-3′-acetoxyacetonyl-3-*p*-methoxyphenyl-2-oxo*cyclo*pentanecarboxylate (IV; R = $CO_2Et$) was isolated in 45% yield. Hydrolysis and reacetylation under the conditions described for the monocyclic analogue failed to yield any decarboxylated product (IV; R = H).

The authors are indebted to Professor G. A. H. Buttle and Dr. P. F. D'Arcy of the School of Pharmacy (University of London) for examining analogues (V) and (VI) for cortical activity. They were tested for their effects on the survival times of adrenalectomised mice subjected to cold stress. Conflicting results were obtained with compound (VI), the initial activity observed not being confirmed by later experiments. Compound

[9] Berlande, *Bull. Soc. chim. France*, 1942, **9**, 644.
[10] Mason and Terry, *J. Amer. Chem. Soc.*, 1940, **62**, 1622.
[11] Richard, *Compt. rend.*, 1935, **200**, 753.
[12] Zaheer *et al.*, *J.*, 1954, 3360; 1955, 1706.

(V) was inactive at a dose of 10 mg./17—20 g. mouse. The very low solubilities of compounds (IV; R = H and $CO_2Et$) in suitable media has so far prevented their biological examination.

## Experimental

1-*Acetoxy*-3-*chloroacetone*.—1 : 3-Dichloroacetone (75 g.) was treated with fused potassium acetate (70 g.) in glacial acetic acid, as described by Hess and Fink.[8] The precipitated potassium chloride was filtered from the rapidly cooled mixture, washed with glacial acetic acid, and the acetic acid distilled from the resulting solution under a reduced pressure of nitrogen. Ether, added to the residue, precipitated further inorganic salts which were filtered off, and the ethereal solution was fractionally distilled under reduced pressure, yielding 1 : 3-dichloroacetone (3·8 g.), b. p. 78—84°/10 mm., 1-acetoxy-3-chloroacetone (30·7 g.), b.p. 100—103°/10 mm., and 1 : 3-diacetoxyacetone (23·7 g.), b. p. 118—122°/8 mm., m. p. 47° [from light petroleum (b. p. 40—60°)] (Found : C, 48·1; H, 5·7. Calc. for $C_7H_{10}O_5$ : C, 48·3; H, 5·75%).

1-*Acetoxy*-3-*iodoacetone*.—1-Acetoxy-3-chloroacetone (5 g.) was added to a solution of anhydrous sodium iodide (6 g.) in dry acetone (25 c.c.), a white precipitate being formed immediately. After being kept at room temperature (12 hr.) the precipitate was filtered off and the acetone solution concentrated under reduced pressure in the cold. Anhydrous ether (50 c.c.) was added to the residue and the resulting precipitate of excess of sodium iodide filtered off. Distillation of the ether *in vacuo*, in the cold, yielded the iodo-compound (8·5 g.), somewhat contaminated with free iodine. Extensive decomposition, with the liberation of large quantities of free iodine, occurred above 60°. The product was used in the crude state in subsequent experiments.

tert.-*Butyl* 2-*Oxo*cyclo*pentanecarboxylate*.—A solution of di-*tert.*-butyl adipate (25·8 g.) [13] in anhydrous benzene (30 c.c.) was added to a stirred, refluxing suspension of sodamide [from sodium (3·3 g.)] in anhydrous benzene (100 c.c.). Stirring and heating were continued until the evolution of ammonia ceased (5 hr.). The semi-solid mixture was cooled and decomposed with ice-cold dilute acetic acid, the benzene layer separated, and the aqueous layer further extracted. Distillation of the washed and dried ($Na_2SO_4$) benzene solution yielded tert.-*butyl* 2-*oxo*cyclo*pentanecarboxylate* (14·7 g.), b. p. 107—109°/14 mm. (Found : C, 64·8; H, 9·1. $C_{10}H_{16}O_3$ requires C, 65·2; H, 8·7%). It yielded a *semicarbazone*, m. p. 155° (from dilute alcohol) (Found : C, 54·5; H, 7·2; N, 17·0. $C_{11}H_{19}O_3N_3$ requires C, 54·8; H, 7·9; N, 17·4%).

A smaller yield of the keto-ester (10 g.) was obtained if powdered sodium (3·3 g.) was used as the condensing agent.

tert.-*Butyl* 1-3′-*Acetoxyacetonyl*-2-*oxo*cyclo*pentanecarboxylate* (VI; R = Bu$^t$).—The above keto-ester (10·65 g.) was added dropwise to a cold, stirred suspension of sodamide [from sodium (1·35 g.)] in anhydrous benzene (125 c.c.) under nitrogen. When the reaction slackened the mixture was heated under reflux until evolution of ammonia ceased (8 hr.). 1-Acetoxy-3-iodoacetone [from chloro-compound (8·8 g.)] was added with ice-cooling. Stirring was continued with cooling (5 hr.) and then at room temperature (16 hr.). The neutral mixture was poured into water (100 c.c.), the benzene layer separated, and the aqueous layer further extracted. The washed and dried ($Na_2SO_4$) benzene solution was distilled under reduced pressure, yielding tert.-*butyl* 1-3′-*acetoxyacetonyl*-2-*oxo*cyclo*pentanecarboxylate* (8·9 g.), b. p. 137—147°/0·04 mm. (Found : C, 60·15; H, 7·65. $C_{15}H_{22}O_6$ requires C, 60·4; H, 7·4%).

Attempted distillation, at 15 mm., of the crude product caused extensive decomposition. Subsequent careful fractionation gave appreciable amounts of 2-3′-*acetoxyacetonyl*cyclo*pentanone*, b. p. 89—96°/0·04 mm. (Found : C, 60·25; H, 7·45. $C_{10}H_{14}O_4$ requires C, 60·4; H, 7·1%), yielding a *disemicarbazone*, m. p. 187° (decomp.) (from 95% alcohol) (Found : C, 46·35; H, 6·8; N, 26·75. $C_{12}H_{20}O_4N_6$ requires C, 46·15; H, 6·4; N, 26·9%).

The corresponding ethyl ester was obtained similarly, except that the sodio-derivative of ethyl 2-oxo*cyclo*pentanecarboxylate (5·15 g.) was prepared by using powdered sodium in boiling benzene. *Ethyl* 1-3′-*acetoxyacetonyl*-2-*oxo*cyclo*pentanecarboxylate* (6·5 g.), b. p. 125—126°/0·05 mm., was obtained (Found : C, 57·9; H, 6·55. $C_{13}H_{18}O_6$ requires C, 57·75; H, 6·65%).

2-3′-*Acetoxyacetonyl*cyclo*pentanone* (V).—(*a*) *tert.*-Butyl 1-3′-acetoxyacetonyl-2-oxo*cyclo*pentanecarboxylate (8·1 g.) and toluene-*p*-sulphonic acid (0·2 g.) in anhydrous benzene (30 c.c.) were heated under reflux until effervescence ceased (3 hr.). The cooled solution was washed with sodium hydrogen carbonate solution and water and dried ($Na_2SO_4$). Distillation under reduced pressure gave 2-3′-acetoxyacetonyl*cyclo*pentanone (3·6 g.), b. p. 92—104°/0·05 mm., yielding a disemicarbazone, m. p. and mixed m. p. 187° (decomp.).

[13] Backer and Homan, *Rec. Trav. chim.*, 1939, **58**, 1048.

(*b*) Ethyl 1-3′-acetoxyacetonyl-2-oxo*cyclo*pentanecarboxylate (3 g.), potassium hydroxide (1·25 g.), and water (12·5 c.c.) were shaken (40 hr.), under nitrogen, at room temperature. Undissolved oil was extracted with ether, the aqueous layer acidified (hydrochloric acid), and the water distilled off under reduced pressure. Acetone-extraction of the residue and subsequent distillation of the acetone gave a residue which was dissolved in 1 : 1 pyridine–acetic anhydride (14 c.c.) and set aside for 24 hr. The solution was diluted with benzene (50 c.c.), washed with water, dried ($Na_2SO_4$), and distilled under reduced pressure, yielding the required diketone (0·6 g.), b. p. 101—104°/0·07 mm. [semicarbazone, m. p. and mixed m. p. 187° (decomp.)].

A similar result was obtained on using aqueous-alcoholic hydrochloric acid at 100°.

*Ethyl γ-Iodobutyrate.*—The procedure [14] for the oxidation of trimethylene chlorohydrin was applied to tetramethylene chlorohydrin.[15] γ-Chlorobutyric acid, b. p. 116—118°/14 mm., was obtained in varying yields (50—77·5%).

Fischer–Speier esterification yielded ethyl γ-chlorobutyrate, b. p. 63—66°/6 mm. (81%).

Later, γ-butyrolactone became available commercially and was readily converted (85% yield) into the desired ester by heating it with absolute alcohol saturated with hydrogen chloride.

The iodo-ester was formed from the chloro-compound by the action of sodium iodide in dry acetone; it had b. p. 94—99°/14 mm.

α-p-*Methoxyphenyladipic Acid.*—Diethyl *p*-methoxyphenylmalonate (100 g.) [16] was added to a stirred solution of sodium ethoxide [from sodium (8·6 g). and absolute alcohol (400 c.c.)] at 60°. The solution was cooled to −15° and ethyl γ-iodobutyrate (160 g.) added rapidly with stirring. Cooling was maintained for a further 6 hr., and the mixture allowed to attain room temperature, stirred for a further 36 hr., and finally heated to 45° (15 min.). The mixture was poured into water and extracted with benzene. Distillation of the dried ($Na_2SO_4$) extract yielded a mixture of triethyl 1-*p*-methoxyphenylbutane-1 : 1 : 4-tricarboxylate and the isomeric diethyl 5-ethoxy-1-*p*-methoxyphenyl-2-oxopentane-1 : 1-dicarboxylate (100 g.), b. p. 155—180°/0·03 mm., having a green fluorescence (Found : C, 63·4; H, 7·0. Calc. for $C_{20}H_{28}O_7$ : C, 63·2; H, 7·4%), together with ethyl γ-iodobutyrate (72 g.) and diethyl *p*-methoxyphenylmalonate (15 g.).

Condensations carried out under reflux gave yields of only 20—27%.

The product was heated under reflux with 10% alcoholic potassium hydroxide (1 l.) for 3 hr., the mixture poured into water (1 l.), sufficient hydrochloric acid added to neutralise the excess of alkali, and the whole evaporated to *ca.* 500 c.c. and strongly acidified. After being kept overnight in the refrigerator the required diacid, together with some potassium chloride, was precipitated. Further evaporation of the mother-liquors gave further yields of product. The dried solid was extracted with hot acetone, evaporation of which yielded crude acid (76·5 g.). Recrystallisation from ethylene dichloride yielded α-p-*methoxyphenyladipic acid* (48·5 g.), m. p. 139—140° (Found : C, 61·8; H, 6·3. $C_{13}H_{16}O_5$ requires C, 61·9; H, 6·35%).

Evaporation of the mother liquors of recrystallisation yielded an acidic oil (27·5 g.) which on re-esterification yielded ethyl *p*-methoxyphenylacetate (6·5 g.) and diethyl *p*-methoxyphenylmalonate (14·5 g.).

*Diethyl* α-p-*Methoxyphenyladipate.*—A solution of the above acid (33 g.) in absolute alcohol (120 c.c.) was saturated with dry hydrogen chloride and heated under reflux (3 hr.). Working up in the usual manner yielded the desired *ester* (38 g.), b. p. 150—152°/0·02 mm. (Found : C, 66·2; H, 7·9. $C_{17}H_{24}O_5$ requires C, 66·2; H, 7·8%).

*Ethyl* 3-p-*Methoxyphenyl-2-oxo*cyclo*pentanecarboxylate* (X; R = H, R′ = Et).—Diethyl α-*p*-methoxyphenyladipate (8 g.) was added to alcohol-free sodium ethoxide [from sodium (1·2 g.)] in anhydrous benzene (90 c.c.), and the mixture heated (3 hr.) under reflux (nitrogen atmosphere). The cooled solution was acidified with acetic acid, washed with water, dilute sodium hydrogen carbonate solution, and water, and dried ($Na_2SO_4$). After distillation of the benzene under nitrogen, the residue was distilled in a short-path still, yielding *ethyl* 3-p-*methoxyphenyl-2-oxo*cyclo*pentanecarboxylate* (4 g.) (bath-temp. 120—140°/1 × $10^{-5}$ mm.) as a greenish-yellow oil (Found : C, 68·5; H, 7·2. $C_{15}H_{18}O_4$ requires C, 68·7; H, 6·9%).

The oil gave a dark violet colour with alcoholic ferric chloride solution and yielded a *semicarbazone*, m. p. 133° (from 40% aqueous alcohol) (Found : C, 60·0; H, 6·3; N, 13·4. $C_{16}H_{21}O_4N_3$ requires C, 60·2; H, 6·6; N, 13·15%).

Powdered sodium was an equally effective cyclising agent. Pure keto-ester was also isolated by extraction of an ethereal solution of the crude product with N-sodium hydroxide, acidification, re-extraction, and evaporation of the dried ether *in vacuo* (Found : C, 68·65, H, 7·05%).

[14] Powell, Huntress, and Hershberg, *Org. Synth.*, 1932, Coll. Vol. I, p. 168.
[15] Star and Hixon, *Org. Synth.*, Coll. Vol. II, p. 571.
[16] Clark and Linnell, *J. Pharm. Pharmacol.*, 1949, **1**, 211.

*Ethyl* 3-p-*Methoxyphenyl*-1-*methyl*-2-*oxo*cyclo*pentanecarboxylate* (X; R = Me, R′ = Et).—The above keto-ester (10 g.), anhydrous benzene (50 c.c.), and powdered sodium (0·9 g.) were stirred and heated under reflux until all the sodium had dissolved (36 hr.). Methyl iodide (5·5 g.) was added dropwise and heating continued (30 hr.) until the mixture was neutral. Working up as above yielded on distillation (bath-temp. 136—140°/1 × $10^{-5}$ mm.) the required methylated *compound* (6·3 g.) (Found: C, 69·85; H, 7·45. $C_{16}H_{20}O_4$ requires C, 69·55; H, 7·25%).

*Reaction between* p-*Methoxyphenylmagnesium Bromide and* 3-*Bromocyclohexene, and Oxidation of the Product.*—3-Bromo*cyclo*hexene [17] (20 g.) was slowly added (1 hr.) to a stirred solution of *p*-methoxyphenylmagnesium bromide [from magnesium (3 g.), *p*-bromoanisole (23·2 g.), and ether (75 c.c.)], at −15°, and the mixture stirred and cooled for a further hour. Working up in the usual manner yielded a *p*-methoxyphenyl*cyclo*hexene (10 g.), b. p. 140°/9 mm. (Found: C, 83·3; H, 8·65. Calc. for $C_{13}H_{16}O$: C, 82·95; H, 8·5%). Ultraviolet absorption (in EtOH): $\lambda_{max.}$ 226 (ε 12,000), 249 (ε 1000), 278 (ε 2300), and 284 mμ. (ε 1900).

Oxidation of the derived *cyclo*hexene (5 g.) with potassium permanganate (11·2 g.) in the presence of magnesium sulphate (4 g.) in aqueous acetone, at room temperature, yielded 2 g. of acid, m. p. 122—128° (Found: equiv., 184, 185. Calc. for $C_{13}H_{18}O_5$: equiv., 126. Calc. for $C_{13}H_{16}O_4$: equiv., 236). Recrystallisation from a variety of sovlents failed to separate the constituent acids.

1-p-*Methoxyphenyl*cyclo*hexene* was obtained as a crystalline material, b. p. 153—156°/10 mm., m. p. 37° [from light petroleum (b. p. 40—60°)] (Braun [18] gives m. p. 35°) (Found: C, 82·5; H, 8·55. Calc. for $C_{13}H_{16}O$: C, 82·95; H, 8·5%), on distillation of the product of reaction between *p*-methoxyphenylmagnesium bromide and *cyclo*hexanone. Ultraviolet absorption (in EtOH): $\lambda_{max.}$ 254 mμ (ε 15,000).

2-p-*Methoxyphenyl*cyclo*hexanone.*—2-Chloro*cyclo*hexanone (15 g.) in anhydrous ether (25 c.c.) was added dropwise to a stirred solution of *p*-methoxyphenylmagnesium bromide [from magnesium (3 g.), *p*-bromoanisole (21·3 g.), and anhydrous ether (45 c.c.)]. The ether was distilled off, anhydrous benzene (60 c.c.) added to the residue, and the mixture heated under reflux (12 hr.) with stirring. The cooled product was poured into water (500 c.c.), the benzene layer separated, and the aqueous layer extracted with benzene. Distillation of the dried ($Na_2SO_4$) benzene solution yielded 2-p-*methoxyphenyl*cyclo*hexanone* (7 g.), b. p. 130—134°/0·05 mm., m. p. 89·5—90° [from light petroleum (b. p. 60—80°)] (Found: C, 76·4; H, 7·85. $C_{13}H_{16}O_2$ requires C, 76·45; H, 7·85%).

δ-p-*Anisoylvaleric Acid.*—To 2-*p*-methoxyphenyl*cyclo*hexanone (1 g.) in acetone at 40° was added an aqueous solution (23 c.c.) of potassium permanganate (1·6 g.) and magnesium sulphate (0·6 g.). After 15 min. the temperature was raised to 60° (maintained for 90 min.). The filtered mixture was evaporated to *ca.* 15 c.c. and acidified with hydrochloric acid. Recrystallisation of the crude product (0·9 g.) from benzene gave δ-*p*-anisoylvaleric acid [19] (0·7 g.), m. p. 127—127·5° (Found: equiv., 236. Calc. for $C_{13}H_{16}O_4$: equiv., 236).

*Bromination of Ethyl* 1-*Methyl*-2-*oxo*cyclo*pentanecarboxylate.*—Ethyl 1-methyl-2-oxo*cyclo*pentanecarboxylate (10 g.) in anhydrous carbon tetrachloride (30 c.c.) was heated under reflux with *N*-bromosuccinimide (10·5 g.), the solution being irradiated with a 100-w lamp. When bromination was complete (18 hr.) the succinimide was filtered off and the filtrate distilled yielding a colourless oil (10·3 g.), b. p. 126—127°/7 mm.

The material coloured quite rapidly on storage under nitrogen at 0°, the analytical figures (Found: C, 45·2; H, 5·4; Br, 29·5. Calc. for $C_9H_{13}O_3Br$: C, 43·4; H, 5·2; Br, 32·1%) indicated, however, that the product was essentially the required ethyl 3-bromo-1-methyl-2-oxo*cyclo*pentanecarboxylate (XI).

*Di*-tert.-*butyl* α-p-*Methoxyphenyladipate.*—α-*p*-Methoxyphenyladipic acid (5·0 g.) and redistilled thionyl chloride (7·2 g.) were heated at 55—60° under reflux until evolution of hydrogen chloride ceased (4 hr.). Excess of thionyl chloride was distilled off under a reduced pressure of dry nitrogen, and the crude acid chloride, dissolved in anhydrous benzene (10 c.c.), added slowly (45 min.) to a stirred, water-cooled mixture of redistilled anhydrous *tert.*-butyl alcohol (7·4 g.) and anhydrous pyridine (4·8 g.). The mixture was heated for 1 hr. at 50°, cooled, washed with 2N-sulphuric acid, sodium hydrogen carbonate solution, and water, and dried ($Na_2SO_4$). The benzene was distilled under reduced pressure, leaving an orange-coloured

[17] Ziegler, Späth, Schaaf, Schumann, and Winkelmann, *Annalen*, 1942, **551**, 80.
[18] Braun, *ibid.*, 1929, **472**, 57.
[19] Fuson, Kuykendall, and Wilhelm, *J. Amer. Chem. Soc.*, 1931, **53**, 4187.

viscous oil (6·1 g.). Distillation (bath-temp. 112—115°/1 × $10^{-5}$ mm.) gave *di*-tert.-*butyl* α-p-*methoxyphenyladipate* (3·3 g.) as a pale greenish solid, m. p. 51° (Found: C, 68·95; H, 8·65. $C_{21}H_{32}O_6$ requires C, 69·25; H, 8·8%).

If the acid chloride–alcohol stage was carried out at 100° for 3 hr. there was a 50% reduction in yield.

Determination of ester value showed the undistilled oil to contain >90% of the desired ester (Found: 91·8 and 91·3% = 5·5 g.), and experiments conducted on the crude material, based on this figure, gave results equivalent to those obtained with the distilled product.

2-3′-*Acetoxyacetonyl*-5-p-*methoxyphenyl*cyclo*pentanone* (IV; R = H).—Di-*tert.*-butyl α-*p*-methoxyphenyladipate (13·3 g.), sodamide [from sodium (1·05 g.)], and anhydrous benzene (100 c.c.) were stirred, under nitrogen, at 45° (3 hr.) and then at 75° (3 hr.), evolution of ammonia then ceasing. 1-Acetoxy-3-iodoacetone [from the chloro-analogue (6·75 g.)] was added to the cooled (ice) solution, and stirring continued, with cooling (3 hr.), and then at room temperature (18 hr.). The neutral mixture was poured into water, the organic layer separated, and the aqueous solution extracted with ether. The combined benzene–ether solutions were extracted with 0·5N-sodium hydroxide, washed with water, and dried ($Na_2SO_4$).

The residue (4·0 g.) after distillation of the solvents was dissolved in anhydrous benzene (40 c.c.), and the solution heated under reflux (3 hr.) with a crystal of toluene-*p*-sulphonic acid. Working up in the normal manner yielded a dark, viscous, non-distillable oil (2·3 g.).

0·7 g. of this material was placed on a 20 × 2 cm. column of neutral alumina. Elution with 4 : 1 benzene–acetone removed a single yellow band. Evaporation of the solvent and short-path distillation (bath-temp. 135°/1 × $10^{-6}$ mm.) of the residue (0·35 g.) gave 2-3′-*acetoxyacetonyl*-5-p-*methoxyphenyl*cyclo*pentanone* (Found: C, 66·7; H, 7·3. $C_{17}H_{20}O_5$ requires C, 67·1; H, 6·6%).

*Ethyl* 1-3′-*Acetoxyacetonyl*-3-p-*methoxyphenyl*-2-*oxo*cyclo*pentanecarboxylate* (X; R = $CH_3$·CO·$CH_2$·OAc, R′ = Et).—Diethyl α-*p*-methoxyphenyladipate (8 g.) was cyclised as described above. To the deep-red solution of the sodium salt so formed was added 1-acetoxy-3-iodoacetone [from the chloro-analogue (3·9 g.)], and the mixture stirred for 3 hr. The neutral mixture was worked up as above, yielding an orange-yellow, viscous oil (6·2 g.), undistillable at 1 × $10^{-6}$ mm. The crude product (0·7 g.) was chromatographed on neutral alumina, 1 : 5 methanol–benzene eluting a single yellow band. Evaporation of the solvents gave ethyl 1-3′-*acetoxyacetonyl*-3-p-*methoxyphenyl*-2-*oxo*cyclo*pentanecarboxylate* (0·5 g.) (Found: C, 64·25; H, 6·6. $C_{20}H_{24}O_7$ requires C, 63·85; H, 6·4%).

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## 241. *Polynuclear Heterocyclic Systems. Part I. The Synthesis of Condensed Glyoxalinium Salts.*

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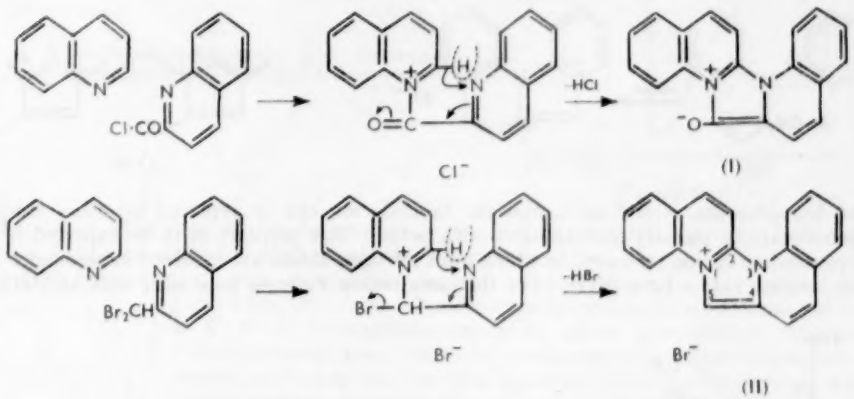
The preparation and properties of pyridino(1′ : 2′-1 : 2)quinolino(1″ : 2″-3 : 4)glyoxalinium, 5-bromopyridino(1′ : 2′-1 : 2)quinolino(1″ : 2″-3 : 4)glyoxalinium, diquinolino(1′ : 2′-1 : 2, 1″ : 2″-3 : 4)glyoxalinium (II), and 5-bromoquinolino(1′ : 2′-3 : 4)*iso*quinolino(1″ : 2″-2 : 1)glyoxalinium salts are described. Catalytic reduction of pyridinoquinolinoglyoxalinium bromide has yielded 3′ : 4′ : 5′ : 6′-tetrahydropyridino(1′ : 2′-1 : 2)quinolino(1″ : 2″-3 : 4)glyoxalinium bromide. Evidence for the structures of these compounds and their relation to Besthorn's Red (I) are discussed.

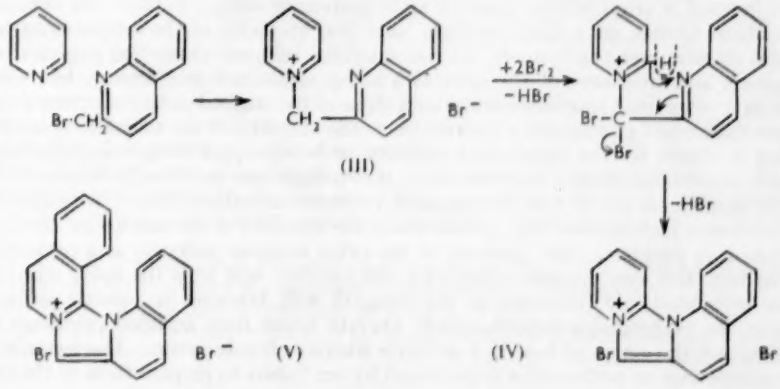
The light-sensitive pigment Besthorn's Red [1,2] is thought to have the mesoionic structure (I).[3,4] The parent heterocyclic skeleton present in this structure, the cationic diquinolino-(1′ : 2′-1 : 2, 1″ : 2″-3 : 4)glyoxalinium (II), has not hitherto been described. The synthesis and study of compounds incorporating this structure would be a valuable step towards a final proof of the structure (I) for Besthorn's Red.

[1] Besthorn and Jaeglé, *Ber.*, 1894, **27**, 907.
[2] Besthorn and Ibele, *Ber.*, 1904, **37**, 1239, 1905, **38**, 2127.
[3] Besthorn, *Ber.*, 1913, **46**, 2762.
[4] Krollpfeiffer and Schneider, *Annalen*, 1937, **530**, 34.

The preparation of Besthorn's Red from quinaldinoyl chloride and quinoline [2,4] probably proceeds by a mechanism of the annexed type. The operation of a similar mechanism should result in the production of cations analogous to that in (II) from the quaternary salts produced by the action of ωω-dibromoquinaldine on pyridine, quinoline, or *iso*quinoline. By this method the pyridino(1′ : 2′-1 : 2)quinolino(1″ : 2″-3 : 4)glyoxalinium (VI) and the diquinolino(1′ : 2′-1 : 2, 1″ : 2″-3 : 4)glyoxalinium (II) salts have been



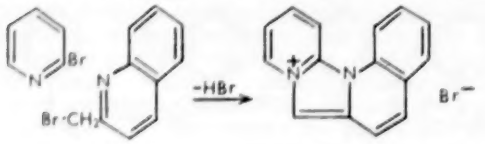

prepared. The use of ω-monobromoquinaldine and pyridine, quinoline, or *iso*quinoline, followed by bromination of the resulting quaternary salts, should yield 5-bromo-derivatives of the glyoxalinium series. This method has yielded 5-bromopyridino(1′ : 2′-1 : 2)quinolino-(1″ : 2″-3 : 4)glyoxalinium (IV) and 5-bromoquinolino(1′ : 2′-3 : 4)*iso*quinolino(1″ : 2″-2 : 1)glyoxalinium (V) salts. The *iso*quinoline compound is formulated thus (V), rather than as 5-bromoquinolino(1′ : 2′-3 : 4)*iso*quinolino(2″ : 3″-1 : 2)glyoxalinium bromide since it is more likely that the 1-position, and not the less reactive 3-position, will be involved in the formation of the glyoxalinium ring. This structure is confirmed by absence of infra-red absorption in the region 860—900 cm.$^{-1}$. This is consistent with the structure (V), but not with the other possible structure which contains isolated aromatic hydrogen atoms.



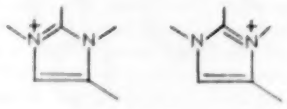

The participation of the α-carbon atom of the pyridine or the second quinoline nucleus in the formation of these compounds has been confirmed by the preparation of the pyridino-(1′ : 2′-1 : 2)quinolino(1″ : 2″-3 : 4)glyoxalinium salt (VI) by an alternative route.

2-Bromopyridine and ω-monobromoquinaldine yielded impure pyridinoquinolinoglyoxalinium bromide, which was converted into the perchlorate and picrate, and their identities were established by infrared comparison with samples of these salt resulting from the reaction between ωω-dibromoquinaldine and pyridine, and by analysis and a mixed melting point of the picrate.

The physical and chemical properties of these compounds are in accord with the condensed glyoxalinium structures deduced from the above methods of synthesis. Thus




(VI)



(VII)

the bromides are typical salts, and the bromide ion can be replaced by other anions (perchlorate or picrate) by treatment with acids. This property is to be expected of a glyoxalinium cation structure in which both nitrogen atoms are involved in resonance of the amidine cation type (VII). For the same reason vigorous treatment with alkylating

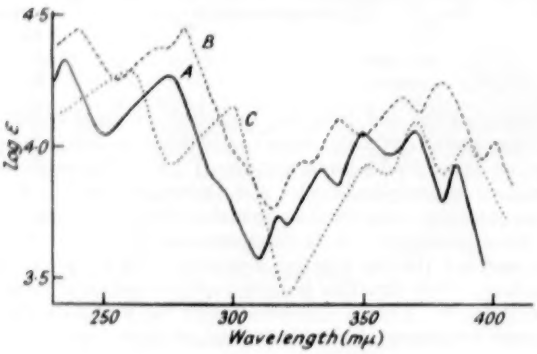



FIG. 1.

*A, Pyridino*(1′ : 2′-1 : 2)*quinolino*(1″ : 2″-3 : 4)*glyoxalinium bromide* (VI) *in water.*

*B, 5-Bromopyridino*(1′ : 2′-1 : 2)*quinolino*(1″ : 2″-3 : 4)*glyoxalinium bromide* (IV) *in water.*

*C, Diquinolino*(1′ : 2′-1 : 2, 1″ : 2″-3 : 4)*glyoxalinium bromide* (II) *in methanol.*

agents (methyl or ethyl halides) does not yield quaternary salts. Further, the salts are not acid salts of amines, since the anhydrous salts (the bromides of the 5-bromo-compounds and the picrates and perchlorates) do not show the infrared absorption expected of the NH group, and treatment of the salts with alkali yields, not free amines, but solutions which have ultraviolet spectra identical with those of the original salts, indicating that they contain the ionised glyoxalinium hydroxides. The stability of the aromatic glyoxalinium nucleus is shown by the substantial stability of 5-bromopyridinoquinolinoglyoxalinium bromide in hot concentrated sulphuric acid; only a slight amount of sulphonation occurred.

The bromides of the 5-bromo-compounds, prepared as outlined above, are isolated from the reaction as perbromides ($Br_3^-$), from which the bromides of the cations are obtained by dissolution in pyridine. The presence of the extra bromine molecule as a perbromide is evident from this easy decomposition with cold pyridine, and from the ready regeneration of the perbromide by treatment of the bromide with bromine in aqueous acetic acid. Further, the perbromides instantaneously liberate iodine from acidified potassium iodide solution, and the action of heat in a dry tube liberates free bromine. The formulation of these compounds as perbromides is confirmed by our failure to prepare them in the absence of the bromide ion.

The ultraviolet spectra and the fluorescent properties of the salts strongly suggest a polycyclic aromatic structure. The close similarity between the spectra of the compounds (Fig. 1) indicates a common structure, and the change from the non-fluorescent

*N*-2-quinolylmethylpyridinium bromide (III) with a final absorption band at 320 mμ to the fluorescent product of its bromination, 5-bromopyridino(1′ : 2′-1 : 2)quinolino(1″ : 2″-3 : 4)glyoxalinium bromide (IV), with a final absorption band at 400 mμ is analogous to the similar differences found between benzene or naphthalene and 1 : 2-benzanthracene.

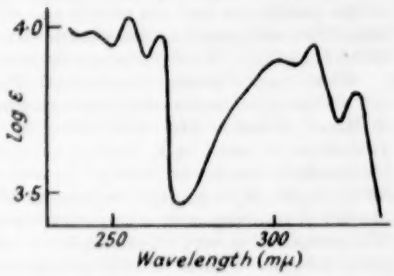
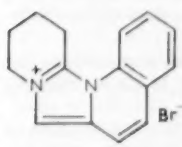



FIG. 2.

*3′ : 4′ : 5′ : 6′-Tetrahydropyridino(1′ : 2′-1 : 2)quinolino-(1″ : 2″-3 : 4)glyoxalinium bromide* (VIII) *in ethanol.*

The strong absorption of the compounds in the region 310—400 mμ (Fig. 1) is best accounted for by structures of the condensed glyoxalinium type.

The behaviour of the compounds towards hydrogenation also supports the polynuclear structures. Pyridinoquinolinoglyoxalinium bromide yielded a 3′ : 4′ : 5′ : 6′-tetrahydro-derivative whose glyoxalinium structure (VIII) is based upon the ready conversion into a perchlorate and a picrate, and upon the fact that aqueous sodium hydroxide causes no visible change, so that the substance is unlikely to be the hydrobromide of an amine. Also, its ultraviolet spectrum (Fig. 2) is intermediate between those of quinolinium and pyridinoquinolinoglyoxalinium salts. This retention of the glyoxalinium structure is not surprising in view of the know reluctance of glyoxalines to undergo hydrogenation.




(VIII)

## EXPERIMENTAL

*Pyridino(1′ : 2′-1 : 2)quinolino(1″ : 2″-3 : 4)glyoxalinium Series* (VI).—(a) *From ωω-dibromoquinaldine and pyridine.* ωω-Dibromoquinaldine (5·0 g.) and pyridine (50 ml.) were boiled under reflux for 2½ hr. The solid which had separated was washed with pyridine, then with ether, and dried. The crude solid (3·4 g.) was treated in aqueous solution with charcoal, then filtered, the filtrate evaporated to dryness, and the solid recrystallised from ethanol–ether. *Pyridino(1′ : 2′-1 : 2)quinolino(1″ : 2″-3 : 4)glyoxalinium bromide hemihydrate* separated as fawn-coloured plates (Found, in material dried at room temperature and pressure: C, 58·3; H, 3·9; N, 8·9. $C_{15}H_{11}N_2Br,\frac{1}{2}H_2O$ requires C, 58·4; H, 3·9; N, 9·1%). The infrared spectrum contains a band at 3450 cm.$^{-1}$ characteristic of the hydroxyl group of the solvent of crystallisation. Light absorption in $H_2O$: max. at 235, 275, 317, 334, 350, 370, and 386 mμ (log ε 4·33, 4·28, 3·73, 3·92, 4·05, 4·07, and 3·94); min. at 250, 310, 320, 340, 360, and 380 mμ (log ε 4·04, 3·58, 3·70, 3·85, 3·96, and 3·79). The anhydrous *bromide* was obtained when the hemihydrate was dried at 100°/0·1 mm. (Found: Br, 27·2. $C_{15}H_{11}N_2Br$ requires Br, 26·8%). The compound is very soluble in water, slightly less soluble in methanol or ethanol, and insoluble in ether or in pyridine. The aqueous solution precipitates silver bromide from aqueous silver nitrate.

Treatment of the bromide in methanol with aqueous perchloric acid yielded the *perchlorate* which separated from methanol as pale yellow needles (Found, in material dried at 100°/0·1 mm.: C, 56·0; H, 3·5. $C_{15}H_{11}O_4N_2Cl$ requires C, 56·5; H, 3·45%).

Prepared from the bromide in ethanol, the *picrate* separated from acetone as yellow plates, m. p. 238—239° (Found: C, 56·8; H, 3·1; N, 15·6. $C_{21}H_{13}O_7N_5$ requires C, 56·4; H, 2·9; N, 15·7%).

(b) *From ω-monobromoquinaldine and 2-bromopyridine.* ω-Monobromoquinaldine (50 mg.), 2-bromopyridine (1·0 g.), and benzene (10 ml.) were boiled under reflux for 30 min. The resulting dark crystals were washed with ether and boiled in aqueous solution with charcoal. Evaporation of the filtrate and crystallisation of the residue from ethanol–ether gave a mixture

of light brown crystals (50 mg.) which could not be separated by further crystallisation. This was probably a mixture of the required bromide with the hydrobromide of α-bromopyridine, the other reaction product. Conversion into the perchlorate, followed by three recrystallisations from methanol, yielded buff needles, which by infrared comparison were proved to be identical with the pyridinoquinolinoglyoxalinium perchlorate prepared as described above. Conversion of this perchlorate into the picrate and crystallisation from acetone yielded yellow plates, m. p. 238—239°, unchanged on admixture with the picrate prepared as described above (Found : C, 56·1 ; H, 2·7%). The infrared curves of the two picrates were identical.

3′ : 4′ : 5′ : 6′-*Tetrahydropyridino*(1′ : 2′-1 : 2)*quinolino*(1″ : 2″-3 : 4)*glyoxalinium Series* (VIII).—Pyridinoquinolinoglyoxalinium bromide (1·0 g.) was hydrogenated for 12 hr. in the presence of Raney nickel at 110—120°/100 atm. The resulting pale yellow solution was filtered and evaporated to small bulk, yielding colourless crystals. Two crystallisations from ethanol gave the *tetrahydro-compound* (0·25 g.) as colourless needles (Found : C, 58·55; H, 5·4; N, 8·4; Br, 24·7. $C_{15}H_{15}N_3Br,\frac{1}{2}C_2H_6O$ requires C, 58·9; H, 5·5; N, 8·6; Br, 24·55%). The presence of alcohol of crystallisation is confirmed by the infrared spectrum (hydroxyl band at 3450 cm.$^{-1}$). The compound is very soluble in water and in polar solvents generally, but is insoluble in non-polar solvents. Silver nitrate precipitates silver bromide from an aqueous solution of the compound. Aqueous sodium hydroxide has no visible action on the salt. Light absorption in EtOH : max. at 245, 255, 265, 300, 312, and 326 mμ (log ε 3·99, 4·03, 3·97, 3·90, 3·94, and 3·80); min. at 240, 250, 260, 270, 308, and 320 mμ (log ε 3·98, 3·94, 3·90, 3·46, 3·88, and 3·71).

Prepared in aqueous methanol and crystallised from methanol the *perchlorate* separated as colourless prismatic needles (Found : C, 55·7; H, 4·8. $C_{15}H_{15}O_4N_3Cl$ requires C, 55·8; H, 4·65%).

Treatment of the bromide in water with picric acid and crystallisation from acetone gave the *picrate* as yellow needles, m. p. 186—187° (Found : C, 55·9; H, 4·15. $C_{21}H_{17}O_7N_6$ requires C, 55·9; H, 3·8%).

5-*Bromopyridino*(1′ : 2′-1 : 2)*quinolino*(1″ : 2″-3 : 4)*glyoxalinium Series* (IV).—A mixture of *N*-2-quinolylmethylpyridinium bromide[5] (2·1 g.), water (10 ml.), acetic acid (50 ml.), and sodium acetate (4·0 g.) was treated at 90° during 10 min. with bromine (3·9 g.) in acetic acid (20 ml.). The yellow crystalline product was separated from the cold mixture, washed with acetic acid, and crystallised from acetic acid, in which it is sparingly soluble, to yield *5-bromopyridinoquinolinoglyoxalinium perbromide* (3·0 g.) as yellow plates, m. p. 340—350° to a red melt (Found : C, 33·8; H, 1·7; Br, 59·4. $C_{15}H_{10}N_3Br_4$ requires C, 33·5; H, 1·85; Br, 59·4%). The product is not very soluble in organic solvents in general, and is only slightly soluble in water from which silver bromide is precipitated by aqueous silver nitrate. A solution of the perbromide in ethanol immediately liberates iodine from acidified starch–potassium iodide paper. The action of heat on the compound liberates free bromine.

The perbromide (1·3 g.) was dissolved in cold pyridine (12 ml.). After a short time a yellow crystalline compound began to be precipitated. When precipitation was complete (several hours) the solid was separated, washed with ether, and crystallised from methanol, to yield yellow needles (0·85 g.) of *5-bromopyridinoquinolinoglyoxalinium bromide*, m. p. 338—345° to a red melt (Found : C, 47·8; H, 2·7; N, 7·3, 7·5; total Br, 42·4, 42·8; $Br^-$, 22·6. $C_{15}H_{10}N_3Br_2$ requires C, 47·6; H, 2·6; N, 7·4; Br, 42·3; $Br^-$, 21·2%). Treatment of the bromide with bromine in aqueous acetic acid regenerated the perbromide whose identity was established by infrared examination. Light absorption in $H_2O$ : max. at 240, 270, 280, 325, 340, 365, 380, and 400 mμ (log ε 4·45, 4·38, 4·45, 3·94, 4·10, 4·19, 4·25, and 4·02); min. at 255, 275, 315, 330, 350, 370, and 395 mμ (log ε 4·25, 4·37, 3·76, 3·92, 4·03, 4·12, and 3·95).

Prepared in methanol and crystallised from ethanol the *perchlorate* was obtained as lemon-yellow crystals (Found : C, 45·55; H, 2·6. $C_{15}H_{10}O_4N_3ClBr$ requires C, 45·3; H, 2·5; Hal, 29·1%). Treatment of the perchlorate with bromine and recrystallisation from aqueous acetic acid left the compound unchanged (Found : C, 45·1; H, 2·7; Hal, 29·7%).

Prepared in ethanol and crystallised from ethanol–acetone the *picrate* was obtained as orange-yellow plates, m. p. 214° (Found : C, 47·7; H, 2·3. $C_{21}H_{13}O_7N_6Br$ requires C, 47·9; H, 2·3%).

*Action of concentrated sulphuric acid on 5-bromopyridinoquinolinoglyoxalinium bromide.* The bromide (0·5 g.) was heated at 100° with concentrated sulphuric acid (2·0 ml.) for 7 hr. Dilution with water (10 ml.) and treatment with hot aqueous picric acid (0·35 g.) yielded an orange solid (0·6 g.), which was washed with water, and alcohol, and dried. A solution of the solid in acetone

[5] Brown, Hammick, and Thewlis, *J.*, 1951, 1145.

was poured on a column of alumina and eluted with acetone (250 ml.), methanol (400 ml.), and finally chloroform (100 ml.). The yellow acetone solution was evaporated and the solid (40 mg.) crystallised from acetone, to yield yellow needles, m. p. 226° (Found : C, 42·0; H, 2·1; N, 11·2. $C_{21}H_{13}O_{10}N_3BrS$ requires C, 41·6; H, 2·0; N, 11·6%). This *picrate* is readily soluble in aqueous sodium carbonate, suggesting that it is a sulphonic acid derivative of the 5-bromopyridinoquinolinoglyoxalinium nucleus.

The orange-yellow methanol eluate was evaporated, and the solid residue (250 mg.) crystallised several times from methanol, and finally from acetone, to yield yellow needles, m. p. 214°, unchanged on admixture with 5-bromopyridinoquinolinoglyoxalinium picrate (Found : C, 47·2; H, 2·6%). The infrared curves of the two picrates were identical.

The eluted chloroform was colourless and yielded no solid on evaporation even though the column remained orange-yellow.

*Diquinolino*(1′ : 2′-1 : 2, 1″ : 2″-3 : 4)*glyoxalinium Series* (II).—ωω-Dibromoquinaldine (4·0 g.) and quinoline (56 ml.) were heated together for 9 hr. just below the b. p. The dark insoluble crystalline solid was separated from the cold mixture, washed with ether, and then with a few drops of methanol–water. The brown solid was heated in aqueous solution with charcoal, and the solution filtered and evaporated to dryness. Crystallisation of the resulting solid from ethanol–ether gave pale yellow prisms (0·8 g.) of *diquinolinoglyoxalinium bromide monohydrate* (Found : C, 61·8; H, 4·2; N, 7·4; Br, 21·8. $C_{19}H_{13}N_3Br,H_2O$ requires C, 62·1; H, 4·1; N, 7·6; Br, 21·8%). The infrared spectrum of the compound shows a band at 3380 cm.$^{-1}$, confirming its formulation as a hydrate. Crystallisation of the compound from water yielded a different *hydrate* as pale yellow needles (Found : C, 55·8, 55·6; H, 5·1, 5·1. $C_{19}H_{13}N_3Br,3\frac{1}{2}H_2O$ requires C, 55·4; H, 4·85%).

Solutions of the salt in polar solvents have a strong blue fluorescence. Light absorption in MeOH : max. at 225, 260, 300, 350, 370, and 390 mμ (log ε 4·12, 4·30, 4·15, 3·94, 4·09, and 4·02); min. at 230, 275, 320, 360, and 380 mμ (log ε 4·11, 3·93, 3·44, 3·88, and 3·89).

The *perchlorate*, prepared in aqueous solution, separated from methanol as yellow needles (Found : C, 61·5; H, 3·6; N, 7·7. $C_{19}H_{13}O_4N_3Cl$ requires C, 61·9; H, 3·6; N, 7·6%).

Prepared in, and crystallised from, ethanol, the *picrate* was obtained as yellow needles, m. p. 261—262° (Found : C, 60·4; H, 3·4. $C_{25}H_{15}O_7N_5$ requires C, 60·4; H, 3·0%).

5-*Bromoquinolino*(1′ : 2′-3 : 4)*isoquinolino*(1″ : 2″-2 : 1)*glyoxalinium Series* (V).—N-2-*Quinolylmethylisoquinolinium bromide*. ω-Monobromoquinaldine (0·3 g.), *iso*quinoline (8·5 ml.), and benzene (10 ml.) were warmed together to effect dissolution. The crystalline solid which separated was washed with ether and crystallised from methanol–ether, yielding the *quaternary salt* (0·5 g.) as colourless plates (Found : Br, 22·0. $C_{19}H_{15}N_2Br,H_2O$ requires Br, 21·7%). The compound is extremely deliquescent.

*Perbromide*. *N*-2-Quinolylmethyl*iso*quinolinium bromide (0·5 g.), acetic acid (2 ml.), water (5 ml.), and sodium acetate (1·0 g.) were heated at 90° and treated dropwise with bromine in acetic acid until no further precipitation was observed. The crystalline solid was separated from the cold mixture, washed with aqueous methanol, then with ether, and crystallised from methanol–ether. 5-*Bromoquinolino*(1′ : 2′-3 : 4)*isoquinolino*(1″ : 2″-2 : 1)*glyoxalinium perbromide* (50 mg.) separated as yellow plates (Found : Br, 54·5. $C_{19}H_{13}N_3Br_4$ requires Br, 54·4%). A solution of the compound in methanol immediately liberates iodine from acidified starch–iodide paper. Light absorption in $H_2O$ : max. at 285, 300, 315, and 370 mμ (log ε 4·21, 4·19 3·97, and 3·91); min. at 295, 310, and 330 mμ (log ε 4·15, 3·94, and 3·55).

Dyson Perrins Laboratory, Oxford University. [*Received, October 4th*, 1955.]

## 242. *The Solubility of Aluminium Chloride in Some Hydrocarbons.*

By Fred Fairbrother, Norman Scott, and Harold Prophet.

A new experimental method has been developed for the measurement of the solubilities of hygroscopic metal halides in non-aqueous solvents. The solubility of anhydrous aluminium chloride in a number of hydrocarbons has been measured in the range 20° to 70°. The results give evidence of a weak complex formation in aromatic hydrocarbons, at room temperature, which disappears as the temperature is raised.

The question of the existence of solution complexes between aromatic hydrocarbons and aluminium halides has, on account of its importance to a knowledge of the mechanism of Friedel–Crafts reactions, attracted the attention of a number of workers as has also the formation of ternary complexes involving also hydrogen halides and giving rise to the so-called "red-oils." The existence of a chemical solvent–solute interaction, in a strictly binary aluminium chloride–hydrocarbon system, involving neither hydrogen chloride nor moisture has hitherto, however, not been satisfactorily established. Even in the case of the more soluble aluminium bromide and benzene, which form a solid complex melting incongruently at 37°,[1] the interaction in solution is so weak as to lead to the conclusion, from the lowering of the vapour pressure of the solvent, that the aluminium bromide in concentrated solution is present mostly as the unsolvated dimer $Al_2Br_6$.[2]

In the case of the much more widely used catalyst, aluminium chloride, a study of complex formation by lowering of vapour pressure or the formation of univariant two-component systems is quite impracticable in hydrocarbon solutions on account of the low solubility. It is well known, however, that such complex formation in solution may be reflected in the temperature dependence of the solubility. We have therefore determined the solubility of aluminium chloride in a number of hydrocarbons, by an analytical technique under very rigidly anhydrous conditions, from 20° to 70°. The results give evidence of weak complex formation at room temperature in aromatic hydrocarbons, which becomes greater as the electron-donating character of the hydrocarbon increases and less as the temperature is raised.

In spite of the obvious relevance to widely-used reactions, few measurements have been made of the solubility of aluminium chloride in hydrocarbons. Menschutkin[3] reported a solubility in benzene of 0·12% at 17° and 0·72% at 80°; also in toluene of 0·26% at 17° and 0·92% at 73°. The context shows that these are in weight per cent of solution. Bruner[4] reported that it is insoluble in benzene and Thomas[5] that it was slightly soluble in benzene and toluene with a small rise of temperature and the production of an olive green or greenish-yellow colour, respectively, and slightly soluble in xylene with a temperature rise of 5° and the production of a heavy red oil after one hour. In none of this previous work does there appear to have been any rigorous exclusion of moisture. The only determinations hitherto carried out under anhydrous conditions are those of Eley and King[6] who measured the solubility in benzene by a synthetical method in closed ampoules from 108·6° to 192·0°. Between these temperatures, which are far above the boiling point of benzene under atmospheric pressure, the solubility is almost ideal and therefore indicates the absence of complex formation in this region. The present results give some indication of an appreciable, though not extensive, complex formation at room temperature even in the case of benzene. In the more donor or "basic" hydrocarbons the chemical interaction is greater, but in all cases as the temperature is raised the complex formation decreases, the solutions becoming regular. For comparison, the solubility

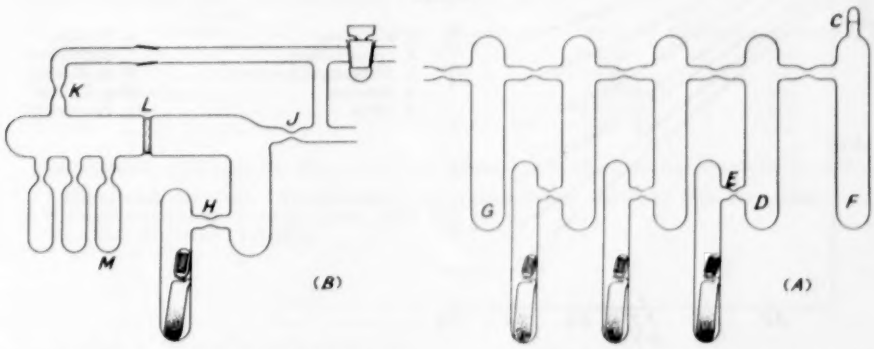
[1] Plotnikov and Gratsianskii, *Chem. Abs.*, 1939, **33**, 2432; Van Dyke, *J. Amer. Chem. Soc.*, 1950, **72**, 3619; Eley and King, *Trans. Faraday Soc.*, 1951, **47**, 1287.
[2] Brown and Wallace, *J. Amer. Chem. Soc.*, 1953, **75**, 6265.
[3] Menschutkin, *J. Russ. Phys. Chem. Soc.*, 1909, **41**, 1089 (*Chem. Zent.*, 1910, **81**, 167).
[4] Bruner, *Z. phys. Chem.*, 1902, **41**, 513.
[5] Thomas, "Anhydrous Aluminium Chloride in Organic Chemistry," Reinhold, New York, 1941, 25.
[6] Eley and King, *Trans. Faraday Soc.*, 1951, **47**, 1287.

has also been measured in *cyclo*hexane, methyl*cyclo*pentane, and *n*-hexane in which no complex formation occurs.

## Experimental

Aluminium chloride was prepared by passing dry hydrogen chloride over high-purity aluminium wire at 400° to 500°, subliming the product four times *in vacuo* and sealing it into fragile hook-ended ampoules. The final product was a pure white solid which could be sublimed without residue. Pure commercial solvents were further purified, fractionated, and dried by appropriate conventional methods. The final drying was in all cases carried out by treatment with aluminium chloride, as described below. The course and success of this drying could be followed by observing the colour of the solutions. A solution of aluminium chloride in dry benzene or toluene is visually quite colourless. When cooled to −195° a lemon-yellow colour is produced, which disappears on warming the solution to room temperature. Solutions in the xylenes and in mesitylene, subjected to the same drying procedure, are lemon-yellow at room temperature and darken on cooling. This behaviour is reminiscent of the colour of iodine in these solvents, which is browner in mesitylene than in benzene and in any aromatic hydrocarbon becomes browner on cooling and more purple on heating, and it points like the case of iodine to the formation of weak complexes between solvent and solute. In both cases the complex formation can be regarded as an " acid–base " or " donor–acceptor " reaction.[7]

Fig. 1.




In the case of an aluminium halide, however, the amount of complex formation with the solvent can be increased by increasing the strength of the " acid " by the addition of hydrogen halide or water. In the first place there is obtained a deeply-coloured ternary complex which may be regarded as $Cl_3AlCl^-..^+HArH$ and gives rise to the so-called " red oils." There is no necessity however, to suppose that colours due to traces of moisture are produced by the formation of the same ternary complexes. These are more probably due to hydrated aluminium chloride–hydrocarbon complexes, $Cl_3AlOH^-..^+HArH$, since they are formed under conditions where hydrolysis is very slow. Such colours, however, are an exceedingly sensitive test for the presence of moisture; *e.g.*, pinholes or minute cracks in the solubility apparatus quickly became evident by the development or deepening of the colour of the solutions. In these cases the results were discarded, although analysis of several such coloured solutions showed only a small difference from the solubility in the colourless solutions.

The solubility apparatus, Fig. 1, made of Pyrex glass, consisted essentially of two parts: the solvent pre-treatment line *A* and the saturation and sampling vessel *B*. Purified solvent was introduced, against a stream of dry nitrogen, through *C* into the previously outgassed apparatus. After removal of any dissolved gases by alternately freezing, pumping, and re-melting, the solvent was frozen at −195°, the apparatus evacuated, and a sample of pure aluminium chloride sublimed into the first pre-treatment tube *D*. After the tube had been sealed-off at *E* the solvent was condensed at −195° on to the aluminium chloride, having been allowed to melt in the first tube *F*, which was then removed. This process was then repeated, three or four such treatments being usually sufficient. The purified frozen solvent was temporarily stored in the tube *G* whilst a sample of pure aluminium chloride was sublimed in a

[7] Fairbrother, *J.*, 1948, 1051.

vacuum into the saturation vessel through *H*, which was then sealed. Finally the purified solvent was condensed on to this aluminium chloride and the apparatus sealed under vacuum at *J* and *K*. The vessel *B* was then suspended in a large water thermostat, controlled at the several temperatures to $\pm > 0{\cdot}1°$, with the longer limb approximately vertical and rocked, for 24 hours at each temperature, about an axis normal to the plane of the diagram. It was then rotated about this axis until the saturated solution filtered through the sintered-glass disc *L* (porosity 3) into the first of the sampling tubes *M*. Only three of these are shown in the diagram: a further set of three was arranged at the rear of the diagram. When sufficient solution had collected in the tube it was sealed off and the sampling side of *B* thoroughly cleaned by condensing small quantities of solvent from the solution, passing it as vapour through the sintered-glass disc, washing it round and returning it as liquid. This was repeated several

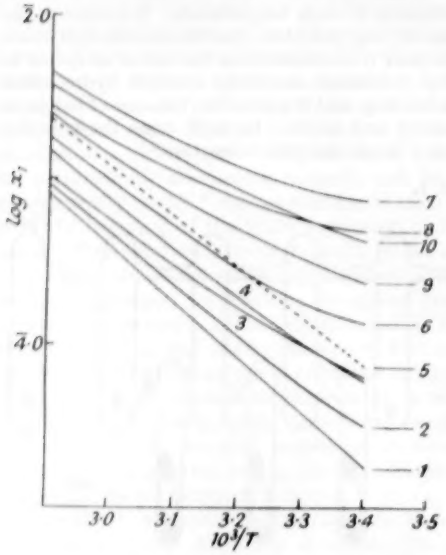



FIG. 2.

1, n-*Hexane*.
2, cyclo*Hexane*.
3, *Methylcyclopentane*.
4, *Benzene*.
5, *Ideal*.
6, *Toluene*.
7, o-*Xylene*.
8, m-*Xylene*.
9, p-*Xylene*.
10, *Mesitylene*.

times. The sample ampoules were weighed, cracked about the middle without separation, and broken under water by gentle shaking. After 2 hr. to ensure complete extraction and hydrolysis, the chloride content was determined by a differential electrometric method based on that of McInnes and Dole.[8] The solubility is given in the Table and plotted as the logarithm of mol. fraction of solute against $1/T$ in Fig. 2.

TABLE. *Solubility* (s) *of aluminium chloride, expressed in mg. of* $Al_2Cl_6$ in 100 g. *of solvent.*

| t° | s | t° | s | t° | s | t° | s | t° | s | t° | s | t° | s |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| n-Hexane | | *cyclo*Hexane | | Methyl*cyclo*-pentane | | Benzene | | Toluene | | *m*-Xylene | | Mesitylene | |
| 20·0 | 5·0 | 20·5 | 9·8 | 20·0 | 19·1 | 19·9 | 20·0 | 20·15 | 39·0 | 19·9 | 113·4 | 21·35 | 98·3 |
| 30·0 | 12·0 | 29·4 | 16·1 | 30·0 | 32·1 | 30·4 | 36·5 | 29·1 | 48·6 | 29·8 | 154·2 | 29·85 | 125·5 |
| 39·8 | 27·3 | 39·4 | 32·2 | 39·8 | 53·9 | 39·8 | 66·7 | 39·1 | 82·7 | 40·2 | 196·8 | 39·65 | 202·5 |
| 49·9 | 56·1 | 45·0 | 51·9 | 49·9 | 96·1 | 49·8 | 118·9 | 49·25 | 139·9 | 50·0 | 283·0 | 59·1 | 478·4 |
| 60·5 | 120·3 | 49·0 | 66·2 | 60·5 | 165·6 | 59·6 | 236·6 | 59·3 | 258·0 | 59·0 | 409·0 | 68·0 | 666·0 |
| 69·8 | 227·7 | 55·0 | 97·8 | 69·8 | 261·2 | 69·4 | 463·0 | 67·6 | 407·6 | 69·1 | 605·2 | | |
| | | 59·8 | 129·7 | | | | | | | | | | |
| | | 67·5 | 240·3 | | | | | | | | | | |

In addition to the figures given, the solubility was also measured in pure *o*-xylene and *p*-xylene. The values were in the same general region as in *m*-xylene, but examination of the solvents by infrared absorption at the end of a run showed that in spite of the very rigorous

[8] McInnes and Dole, *J. Amer. Chem. Soc.*, 1929, **51**, 1119.

drying, considerable (15 to 20%) isomerisation into *m*-xylene had occurred. Even in the case of *m*-xylene, more trouble was experienced with oil formation in the pre-treatment and with reproducibilty of results than with the other solvents, and the final results still show a small scatter about a smooth curve. There was, however, much less isomerisation, amounting to less than 5% after 6 days and a maximum temperature of 70°.

The ideal solubility curve of Fig. 2 has been calculated by the equation given by Hildebrand:[9]

$$\log_{10} x_1 = \frac{-\Delta H_{T_M}}{4{\cdot}575} \cdot \frac{(T_M - T)}{T_M \cdot T} + \frac{\Delta C_p}{4{\cdot}575} \cdot \frac{T_M - T}{T} - \frac{\Delta C_p}{1{\cdot}987} \log_{10} \frac{T_M}{T}$$

where $x_1$ = mol. fraction of solute (ideal); $\Delta H_{T_M}$, heat of fusion, = 17·0 kcal. at m.p. $T_M = 466°$K;[10] $\Delta C_p$ = molar heat capacity change of solute on fusion = $35{\cdot}9 - 0{\cdot}056T$ kcal.[11]

The curves for the saturated hydrocarbons and for benzene lie below the ideal solubility, as does also that for toluene at the higher temperatures. At the lower temperatures there is a general increase of solubility as the donor character of the hydrocarbons becomes greater, though there is little difference between the solubility in *m*-xylene and in mesitylene. There is little evidence of complex formation at the higher temperatures, where all form regular solutions. The solute parameters of aluminium chloride have been calculated, according to Hildebrand's method,[12] from the solubilities at 65° (interpolated), using solvent parameters obtained by interpolation from the data given by Hildebrand and Scott and $(\Delta E/V)^{\frac{1}{2}} = 7{\cdot}3$ for methyl*cyclo*pentane. The results are as follows:

| Solvent | Solvent parameter | $10^2x$ | Solute parameter, $Al_2Cl_6$ |
|---|---|---|---|
| *n*-Hexane | 6·7 | 0·525 | 8·9 |
| *cyclo*Hexane | 7·6 | 0·603 | 9·6 |
| Methyl*cyclo*pentane | 7·3 | 0·679 | 9·2 |
| Benzene | 8·5 | 1·00 | 9·8 |
| Toluene | 8·4 | 1·23 | 9·4 |

DEPARTMENT OF CHEMISTRY, THE UNIVERSITY, MANCHESTER, 13. [*Received, November 7th*, 1955.]

[9] Hildebrand and Scott, "The Solubility of Non-electrolytes," Reinhold, New York, 1950.
[10] Fisher and Rahlfs, *Z. anorg. Chem.*, 1932, **205**, 1.
[11] Landolt Börnstein, Tabellen.
[12] Ref. 9, p. 275.

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## 243. *Saponins and Sapogenins. Part IV.* Agapanthagenin (22a-Spirostan-2α : 3β : 5α-triol), a New Sapogenin from* Agapanthus *Species.*

By (MRS.) T. STEPHEN.

From several *Agapanthus* species a small quantity of yuccagenin (22a-spirost-5-en-2α : 3β-diol) has been isolated whilst the main constituent is a new steroidal sapogenin, $C_{27}H_{44}O_5$, for which the name agapanthagenin is proposed. The compound is considered to be 22a-spirostan-2α : 3β : 5α-triol.

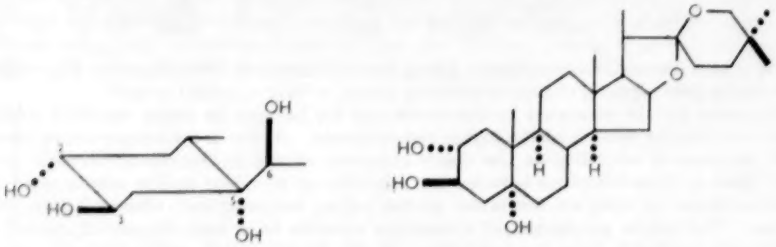
AGAPANTHAGENIN has three hydroxyl groups, one of which is considered to be tertiary since only a diacetate is formed, and this contains a free hydroxyl group. The infrared absorption spectrum of the diacetate shows agapanthagenin to be a true steroidal sapogenin with an *iso*-configuration of ring F. The acetate band at 1244 cm.$^{-1}$ closely resembles that recorded [1] for gitogenin diacetate (5α : 22a-spirostan-2α : 3β-diol diacetate) and, in addition to indicating the two hydroxyl groups to be at $C_{(2)}$ and $C_{(3)}$, the simplicity of this band suggests a *trans*-fusion of rings A and B. Agapanthagenin and its diacetate are saturated (tetranitromethane test and the ultraviolet absorption spectrum [2]) and in boiling 2N-ethanolic hydrochloric acid develop a strong green-yellow fluorescence indicative of

* Part III, *Chem. and Ind.*, 1954, 727.

[1] Eddy, Wall, and Scott, *Analyt. Chem.*, 1953, **25**, 266.
[2] Bladon, Henbest, and Wood, *J.*, 1952, 2737.

dehydration [3] and yield a crystalline compound $C_{54}H_{86}O_9$. Boiling 1N-ethanolic hydrochloric acid hydrolyses the diacetate to agapanthagenin.

Analyses of the compound $C_{54}H_{86}O_9$ agree with its formulation as an equimolar complex of agapanthagenin and its dehydration product. The formation of stable molecular complexes in the steroid field is well established.[4] Chromatographic analysis of the complex failed to separate it into its components, but acetylation and fractional crystallis-




ation gave agapanthagenin and yuccagenin diacetates. The complex and yuccagenin diacetate derived from agapanthagenin are both identical with the corresponding compouunds isolated from the plant, and the infrared absorption spectrum of yuccagenin diacetate agrees with that recorded by Eddy, Wall, and Scott.[1] Chromatographic analysis of the acetylated complex gave (*a*) an isomer of yuccagenin diacetate, indicting that a shift of the double bond may have taken place, and (*b*) agapanthagenin diacetate. Agapanthagenin diacetate may be dehydrated directly to yuccagenin diacetate thus indicating that the free hydroxyl group takes part in the dehydration; being tertiary in character this hydroxyl group must be situated at $C_{(5)}$. The specific spatial configuration of this group is derived by comparing the dehydration of agapanthagenin with the behaviour of the two isomeric ergostadiene-3 : 5 : 6-triols.[5] Triol I and hydroxyergostanedione, in which rings A and B have a *trans*-decalin configuration, are readily dehydrated with hydrogen chloride; whereas triol II which is a *cis*-decalin derivative does not lose water even on prolonged treatment of its diacetate with phosphoric oxide. Petrow, Rosenheim, and Starling [6] have likewise shown that 3β : 6β-diacetoxycholestan-5α-ol readily loses water, yielding cholest-4-ene-3β : 6β-diol diacetate. That the triol II could not be dehydrated can only be attributed to the specific spatial configuration of the tertiary $C_{(5)}$-hydroxyl group in *cis*-decalin derivatives. The assignment of 22a-spirostan-2α : 3β : 5α-triol to agapanthagenin receives further support from the ready dehydration which is to be expected if rings A and B are *trans*-fused. The $C_{(5)}$-hydroxyl group is then polar and consequently can be eliminated with the coplanar $C_{(6)}$-hydrogen.

## Experimental

Rotations were determined in chloroform at 25°. M. p.s were determined on a block and are corrected. The alumina used for chromatography was washed and activated at 180°.

*Isolation of Sapogenins.*—Minced, fresh *Agapanthus* rhizomes (1000 g.) and 2N-hydrochloric acid (1000 c.c.) were boiled for 4 hr. The hydrolysed saponins and the residual plant material were filtered off, washed free from acid, and dried at 100° for 72 hr. The dried cake was ground and extracted continuously for 6 hr. with boiling carbon tetrachloride (250 c.c.). Excess of solvent was removed and the residual material washed free from fatty impurities with ether to give a mixture of sapogenins (4 g.), m. p. 200—250°. This mixture was chromatographed in benzene containing 20% of chloroform on a column of alumina. Elution with benzene–chloroform or chloroform gave, no sapogenin, but chloroform containing ½% of methanol eluted yuccagenin–agapanthagenin *complex* ($C_{54}H_{86}O_9$) (5—10% of the original mixture), which

[3] Whitby, *Biochem. J.*, 1923, **17**, 5; Schoenheimer, Dam, and von Gotberg, *J. Biol. Chem.*, 1953, **110**, 659.
[4] Spring and Swain, *J.*, 1943, 613; Plattner, Petrzilka, and Lang, *Helv. Chim. Acta*, 1944, **27**, 513.
[5] Dunn, Heilbron, Phipers, and Samant, *J.*, 1934, 1576.
[6] Petrow, Rosenheim, and Starling, *J.*, 1938, 677.

formed needles (from ethanol), m. p. 246—248°, $[\alpha]_D$ −171° (*c*, 1·6) (Found: C, 73·7; H, 9·8%; active hydrogen, 5. $C_{54}H_{86}O_9$ requires C, 73·8; H, 9·7%; active hydrogen, 5). Elution with chloroform containing increasing proportions of methanol furnished, after all the complex had been removed, small amounts of agapanthagenin (22a-spirostan-2α : 3β : 5α-triol); but this is so teniously held on alumina that elution is unsatisfactory. It can all be removed by treatment with boiling water, evaporation of the water, and extraction of the agapanthagenin with boiling carbon tetrachloride. *Agapanthagenin* crystallises from ethyl methyl ketone as plates, m. p. 285° (Found: C, 71·95; H, 10·0. $C_{27}H_{44}O_5$ requires C, 72·3; H, 9·8%). Light absorption in ethanol: inflections at 2050, 2100, and 2250 Å; ε 132, 59, and 18; it thus shows no region of maximal absorption associated with ethylenic centres. The specific rotation in chloroform and active hydrogen could not be determined because of its sparing solubility.

*Agapanthagenin Diacetate* (2α : 3β-*Diacetoxy*-22a-*spirostan*-5α-*ol*).—Agapanthagenin (2 g.) was acetylated with pyridine (7·5 c.c.) and acetic anhydride (3 c.c.); the *diacetate* (2 g.) crystallised from ethanol in needles, m. p. 298—299°, $[\alpha]_D$ −101° (*c*, 3·5) (Found: C, 70·0; H, 9·1; Ac, 16·1%; active hydrogen, 1. $C_{31}H_{48}O_7$ requires C, 69·9; H, 9·0; Ac, 16·1%; active hydrogen, 1). The infrared absorption spectrum in carbon disulphide showed the usual four bands, at 865, 900, 920, and 981 cm.$^{-1}$, the 900 band being stronger than the 920 as normally associated with the *iso*-configuration of ring F. The acetate band at 1244 cm.$^{-1}$ is typical of sapogenin diacetates and closely resembles that of gitogenin diacetate (2α : 3β-diacetoxy-5α : 22a-spirostan).[1] Light absorption in ethanol: inflections 2050, 2100, and 2250 Å; ε 410, 288, and 188. There was no region of maximal absorption associable with ethylenic centres.

*Yuccagenin Diacetate* (22a-*Spirost*-5-*en*-2α : 3β-*diol Diacetate*).—Acetylation of the agapanthagenin–yuccagenin complex (2 g.) with pyridine and acetic anhydride yielded a mixture (2 g.), m. p. 160—200°. Rapid fractional crystallisation of this from ethanol caused yuccagenin diacetate to separate first, and the agapanthagenin diacetate was purified separately (from ethanol) and found to be identical with that described above. The yuccagenin diacetate was crystallised from light petroleum (b. p. 40—60°), forming needles, m. p. 178°, $[\alpha]_D$ −130° (*c*, 2·8) (Calc. $[\alpha]_D$ −139°) (Found: C, 72·3; H, 9·0; Ac, 16·8. Calc. for $C_{31}H_{46}O_8$: C, 72·4; H, 8·9; Ac, 16·7%). The infrared absorption spectrum in carbon disulphide agreed with that recorded for yuccagenin diacetate.[1]

Chromatographic separation of the acetylated complex on alumina with light petroleum as solvent and 40% benzene–petroleum as eluant gave an *isomer* of yuccagenin diacetate. This crystallised from light petroleum as needles, m. p. 211°, $[\alpha]_D$ −112° (*c*, 2·5) (Found: C, 72·3; H, 8·85; Ac, 16·8. Calc. for $C_{31}H_{46}O_8$: C, 72·4; H, 8·9; Ac, 16·7%). The m. p. of a mixture with yuccagenin diacetate was 170—190°. Elution of the column with 10% of chloroform in benzene liberated agapanthagenin diacetate.

*Yuccagenin.* (22a-*Spirost*-5-*en*-2α : 3β-*diol*).—Yuccagenin diacetate was hydrolysed according to Perkin's procedure and yielded yuccagenin as needles (from ethanol), m. p. 248°, $[\alpha]_D$ −122° (*c*, 1·6) (Found: C, 75·4; H, 9·9. Calc. for $C_{27}H_{42}O_4$: C, 75·35; H, 9·8%).

*Agapanthagenin–Yuccagenin Complex.* ($C_{54}H_{86}O_9$).—Agapanthagenin or its diacetate was refluxed in 2N-alcoholic hydrochloric acid for 5 hr., the solution acquiring a deep greenish-yellow fluorescence. The complex produced crystallised from alcohol in needles, m. p. 246—248°, $[\alpha]_D$ −171° (*c*, 1·5) (Found: C, 73·7; H, 9·8%; active hydrogen, 5), and was identical with that obtained directly from the plant. It was similarly resolved on acetylation into yuccagenin and agapanthagenin diacetates.

*Dehydration of Agapanthagenin Diacetate.*—To agapanthagenin diacetate (2 g.) in dry pyridine (5 c.c.) at 20° thionyl chloride (1 c.c.) in pyridine (5 c.c.) was added dropwise with shaking. After 2 hr. pyridine hydrochloride had separated; dilute hydrochloric acid was added, and the product was extracted with ether. The ethereal solution was washed and dried, and the product crystallised several times from alcohol to give yuccagenin diacetate, identical with that previously obtained.

The author thanks Professor F. L. Warren and Professor H. Stephen for their interest.

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## 244. *Raman Effect of the Borohydride Ion in Liquid Ammonia.*

By L. A. Woodward and H. L. Roberts.

The Raman spectra of solutions of potassium borohydride in liquid ammonia at approximately −40° show, in addition to the frequencies of the solvent, a single polarized shift, $\Delta\nu = 2270 \pm 3$ cm.$^{-1}$, which is attributed to the totally symmetric vibration $\nu_1$ of the regular tetrahedral borohydride ($BH_4^-$) ion. It is pointed out that, for the isoelectronic species $BH_4^-$, $CH_4$, and $NH_4^+$, the bond-stretching force constant increases in the order given, the increase in going from borohydride ion to methane being markedly greater than that in going from methane to ammonium ion.

The species borohydride ion, methane, ammonium ion form a simple isoelectronic set, the study of which may give evidence on the question of the dependence of bond properties (force constants, etc.) upon the charge of the central nucleus. Only for methane and ammonium ion have Raman spectra been reported. We have now investigated the borohydride ion in solutions of the potassium salt in liquid ammonia. The choice of solvent was dictated by the instability of the ion in water.

We have observed, in addition to the known spectrum of the solvent, one Raman frequency, $\Delta\nu = 2270$ cm.$^{-1}$, excited both by Hg 4358 Å and by Hg 4046 Å lines. Our experiments show that this frequency is polarized; and it may therefore be confidently assigned to the symmetrical "breathing" frequency $\nu_1(A_1)$ of the regular tetrahedral ion, $BH_4^-$.

The infrared spectrum of the borohydride ion in solid sodium borohydride, as reported by Price,[1] shows two absorptions: one fairly sharp at 1080 cm.$^{-1}$ and the other a broader band centred at 2270 cm.$^{-1}$. Clearly these are to be identified with the only two infrared-active fundamentals $\nu_4(F_2)$ and $\nu_3(F_2)$, respectively. The corresponding values for the borohydride ion in the lithium salt are [1] 1096 and 2320 cm.$^{-1}$. Frequencies obtained from crystalline salts will be expected to differ somewhat from those for the ion in solution; but it is clear that $\nu_3$ and $\nu_1$ are approximately equal. This is not unexpected in view of the large mass of the boron atom as compared with that of the hydrogen atom. According to the simple valency force field for a regular tetrahedral species, $XY_4$, $\nu_3$ is always greater than $\nu_1$ but approaches equality with it as the mass ratio $m_X/m_Y$ becomes larger. Observations for a range of molecules and ions bear this out quite well; but it is found that $\nu_3$ may even become slightly less than $\nu_1$ when $m_X/m_Y$ is large, *e.g.*[2] for $AlH_4^-$.

The approximate equality of $\nu_3$ and $\nu_1$ for the borohydride ion probably makes it impossible to observe the former as a distinct Raman line. It may be noteworthy that, although our experiments were not capable of giving the degree of depolarization exactly, they suggest that $\Delta\nu$ 2270 cm.$^{-1}$ is not completely polarized. This would be in accord with its being mainly due to the completely polarized $\nu_1$ but containing also a contribution from the depolarized shift $\nu_3$.

The other two fundamentals, $\nu_2$ and $\nu_4$, are also permitted in the Raman effect; but they are expected to be very weak, and it is not surprising that we have not observed them. Neither $\nu_2$ nor $\nu_4$ has been observed for the isoelectronic ammonium ion in solution, despite the facts that the concentration of this species in water can be made much greater than for the borohydride ion in liquid ammonia, and that aqueous solutions are much easier to investigate. So also for the isoelectronic methane molecule, $\nu_2$ and $\nu_4$ appear only very feebly even for liquid methane,[3] while $\nu_4$ has never been detected for the gas. The feebleness of these two frequencies should therefore make their observation for the borohydride ion very difficult; but in addition, the value of $\nu_4 = 1080$ from the infrared spectrum is such that its Raman line would be masked by the weak band of liquid ammonia at 1075—1090 cm.$^{-1}$. As to $\nu_2$, which is inactive in infrared absorption, we may use the other three frequencies and calculate an approximate value for it by means of a three-parameter force

[1] Price, *J. Chem. Phys.*, 1949, **17**, 1044.
[2] Lippincott, *ibid.*, p. 1351.
[3] Crawford, Welsh, and Harold, *Canad. J. Phys.*, 1952, **30**, 81.

field. Using the orbital valency field with repulsion between non-bonded atoms [4] and taking the exponent $n$ in the repulsion term as equal to 7, we obtain $\nu_2 = 1065$ cm.$^{-1}$. Thus $\nu_2$ is approximately equal to $\nu_4$, as well as $\nu_3$ being approximately equal to $\nu_1$. The frequencies for the borohydride ion are given in Table 1, together with those of the isoelectronic methane molecule and ammonium ion (see Herzberg [5]). It is seen that (i) each

TABLE 1. *Vibrational frequencies of isoelectronic species.*

| | $\nu_1$ ($A_1$) | $\nu_2$ ($E$) | $\nu_3$ ($F_2$) | $\nu_4$ ($F_2$) |
|---|---|---|---|---|
| $BH_4^-$ | 2270 | (1065 calc.) | 2270 | 1080 |
| $CH_4$ | 2914 | 1526 | 3020 | 1306 |
| $NH_4^+$ | 3033 | 1685 | 3134 | 1397 |

of the frequencies increases regularly from $BH_4^-$ to $NH_4^+$, *i.e.* as the central positive charge increases; and (ii) for each frequency the increase from $BH_4^-$ to $CH_4$ is considerably greater than that from $CH_4$ to $NH_4^+$.

The changes of the frequencies shown in Table 1 imply corresponding changes in the force constants. Confining our attention to the "breathing" frequencies, $\nu_1$, and using the simple valency force field (without repulsion term) as a sufficiently good approximation for the purposes of this discussion, we obtain the values of the bond-stretching force constant $k$ given in Table 2.

TABLE 2. *Stretching force constants of isoelectronic species.*

| | $BH_4^-$ | $CH_4$ | $NH_4^+$ | $AlCl_4^-$ | $SiCl_4$ | $PCl_4^+$ |
|---|---|---|---|---|---|---|
| $\nu_1$ (cm.$^{-1}$) | 2270 | 2914 | 3033 | 349 | 424 | 451 |
| $10^{-5}k$ (dynes/cm.) | 3·06 | 5·04 | 5·46 | 2·54 | 3·75 | 4·24 |
| % increase of $k$ relative to preceding value | — | 64 | 8 | — | 48 | 13 |

It is seen that the percentage increase of $k$ in going from $BH_4^-$ to $CH_4$ is markedly greater than that in going from $CH_4$ to $NH_4^+$. This may be connected with the fact that the percentage increase of the central positive charge in going from boron to carbon (20 reckoned on atomic numbers or 33·3 if the two 1s electrons are included) is greater than that in going from carbon to nitrogen (16·7 or 25, respectively).

The only other analogous isoelectronic set for which data are available appears to be $AlCl_4^-$, $SiCl_4$, and $PCl_4^+$. The first of these has been investigated by Gerding and Houtgraaf.[6] The species $PCl_4^+$, which occurs with $PCl_6^-$ in solid phosphorus pentachloride, has been recently studied by the same workers.[7] For data for $SiCl_4$ see Herzberg.[5] The values of $\nu_1$ and the stretching force constants $k$ are included in Table 2. From a comparison with the corresponding data for $BH_4^-$, $CH_4$, and $NH_4^+$ it is seen that both isoelectronic sets show very similar features. In particular, we note the much larger percentage increase of $k$ in going from the negative ion to the neutral molecule, as compared with going from the latter to the positively charged ion.

## EXPERIMENTAL AND RESULTS

*Purification of Materials.*—The ammonia was generated from the solution, $d$ 0·88, dried, and liquefied by cooling to −80°. It was purified by distillation *in vacuo*. The sample of potassium borohydride used was placed at our disposal by Dr. R. E. Richards. Originally supplied by May and Baker, it had been purified by repeated recrystallisation from liquid ammonia.

*Preparation of Solutions.*—Liquid ammonia was distilled on to solid potassium borohydride *in vacuo* and left a sufficient time at *ca.* −60°. The solution so obtained was filtered through a sintered-glass disc at −60° in a suitable all-glass apparatus, care being taken to avoid contact with grease. Finally the solution was poured into the pre-cooled Raman tube through a pre-cooled funnel. Evaporation of ammonia during this process was negligible. The concentration of the solutions used was about 2M.

[4] Heath and Linnett, *Trans. Faraday Soc.*, 1948, **44**, 561, 878.
[5] Herzberg, "Molecular Spectra and Molecular Structure," Van Nostrand, New York, 1945, Vol. 2, p. 167.
[6] Gerding and Houtgraaf, *Rec. Trav. chim.*, 1953, **72**, 21.
[7] *Idem, ibid.*, 1955, **74**, 5.

*Photographing of Raman Spectra.*—The apparatus was that previously described by Rolfe and Woodward [8] (Toronto-arc excitation, Hilger E 518 spectrograph), slightly modified to take a Raman tube of about 4 ml. capacity. Excellent spectra were obtained with exposure times up to 2 hr., the sample being maintained throughout at approximately −40° by a stream of cold air passing between the Raman tube and a surrounding unsilvered Dewar sleeve. The plates were Kodak Special Scientific (emulsion Oa, sensitivity G). The use of a primary filter of Rhodamine 6 G.D.N. extra was found to be advantageous. The state of polarization of the Raman lines was determined qualitatively by the method of polarized incident light, suitable polaroid cylinders surrounding the Raman tube being used.

*Results.*—The following Raman spectrum was observed for the solvent, both alone and in the solutions : 1075—1090 cm.$^{-1}$ (weak band), 3214 cm.$^{-1}$ (very strong, polarized), 3298 (very strong, polarized), and 3390 (very strong, depolarized). The weak band was excited by the Hg 4358 Å line only; the other shifts were excited by both Hg 4358 and Hg 4046 Å irradiations. The frequencies were measured in the usual manner, a copper arc being used as standard. The limits of error are estimated to be ±3 cm.$^{-1}$ for strong lines. The results are in agreement with those of previous workers.[9]

For the solutions of potassium borohydride we observed one Raman frequency, excited by the Hg 4358 Å (medium intensity) and also by the Hg 4046 Å line (weak). The measured $\Delta\nu$ is 2270 cm.$^{-1}$. This frequency was absent for the solvent alone. Spectrograms taken with polarized incident light showed clearly that the line is polarized, but suggest that the degree of polarization is probably greater than zero.

We thank Dr. R. E. Richards for kindly placing at our disposal the sample of purified potassium borohydride, and for advice on the manipulation of liquid ammonia solutions. We are indebted to the Royal Society for a grant (to L. A. W.) which covered the cost of purchasing the spectrograph. One of us (H. L. R.) thanks the D.S.I.R. for a maintenance grant.

INORGANIC CHEMISTRY LABORATORY, OXFORD. [*Received, November 23rd,* 1955.]

[8] Rolfe and Woodward, *Trans. Faraday Soc.*, 1954, **50**, 1030.
[9] Kinumaki and Aida, *Sci. Reports Tohuku Univ.*, 1954, *A*, **6**, 186.

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## 245. *Theoretical Studies of the Macrocyclic Pigments. Part I. The Structure of Bacteriochlorophyll.*

By J. R. BARNARD and L. M. JACKMAN.

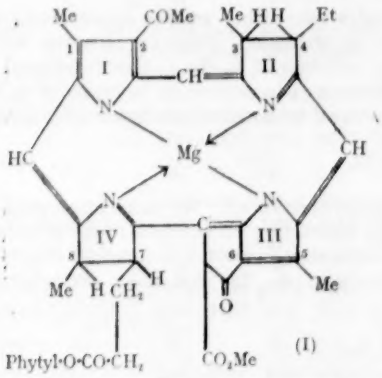
Molecular-orbital calculations have been performed on chlorin and a tetrahydroporphin of $C_{2v}$ symmetry. It is found that the predicted centres of gravity for the longest-wavelength transitions lie in the order $C_{2v}$ tetrahydroporphin < porphin < chlorin < $D_{2h}$ tetrahydroporphin. This is in agreement with spectra in the tetraphenylporphin series, where the porphin and the chlorin lie between two tetrahydro-compounds of unknown structure. It follows that bacteriochlorophyll is related to the tetrahydroporphin of $D_{2h}$ symmetry in which the two pairs of hydrogen atoms are located on opposite pyrrole rings. This effect of symmetry is also observed in the polycyclic aromatic hydrocarbons related to coronene.

THERE are only two structural features in which bacteriochlorophyll differs from chlorophyll-*a*. This close relation is exemplified by the conversion of the former into 2-acetyl-2-devinylphæophorbide-*a* which has been synthesised from chlorin-$e_6$.[1] The degradation is effected by the removal of the magnesium atom, the phytyl residue, and the (? two) extra hydrogen atoms.[2] Bacteriochlorophyll has an acetyl group at position 2 and differs in its state of hydrogenation. The preservation of long-wavelength absorption (*ca.* 7500 Å)

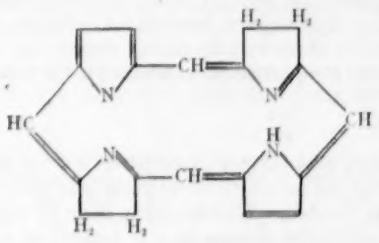
[1] Cf. Stoll and Wiedemann, *Fortschr. chem. Forsch.*, 1952, **2**, 538 and references there cited.
[2] Fischer, Lambrecht, and Mittenzwei, *Z. physiol. Chemie*, 1938, **253**, 1.

and the ease of dehydrogenation [3] suggest that the two extra hydrogen atoms are located on one of the three pairs of peripheral or β-positions of ring I, II, or III (see structure I).* Thus, apart from the stereochemistry of the various asymmetric centres, the only outstanding problem regarding the structure of bacteriochlorophyll is the decision between these three pairs of positions.

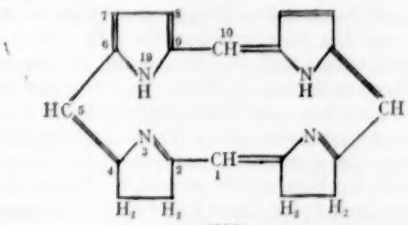
Mittenzwei [3] has suggested that ring II carries the extra hydrogen atoms and that bacteriochlorophyll has the structure (I). He has shown that oxidative degradation of bacteriochlorophyll derivatives yields small quantities of an oil which was possibly ethylmethylsuccinic anhydride. As this compound is not produced when chlorophyll derivatives are similarly degraded Mittenzwei claims that it must have arisen from a hydrogenated ring II. Seely [5] recently criticised the significance of these findings on the grounds that the failure of chlorophyll derivatives to yield ethylmethylsuccinic anhydride does not exclude the possibility of its having arisen from ring IV by decarboxylation before degradation of the bacteriochlorophyll derivatives. Seely suggested that the extra hydrogen atoms may be in either ring I or ring III, probably the latter.



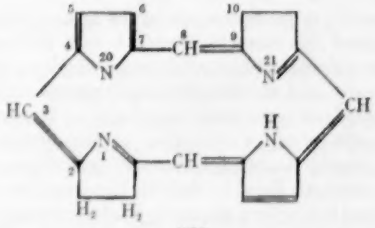

(I)



(II)



(III)



(IV)

We agree with Seely that Mittenzwei's proof of structure is by no means rigid. We now provide, by the application of the empirical L.C.A.O. M.O. method, some support for the correctness of structure (I).† Our studies suggest marked spectral differences for the two tetrahydroporphin models (II) and (III) which, if they exist, would allow an unambiguous

* There is now conclusive evidence that ring IV in chlorophyll has a 3 : 4-dihydropyrrole structure.[4]

† Professor Longuet-Higgins (personal communication) has stressed the fact that too much reliance should not be placed on simple M.O. methods when applied to the calculation of transition energies and that more refined calculations using self-consistent orbitals would be better adapted for this purpose. We are undertaking a more detailed treatment.

[3] Mittenzwei, *ibid.*, 1942, **275**, 93.
[4] Ficken, Johns, and Linstead, Chem. Soc. Spec. Publ. No. 3, 1955.
[5] Seely, U.S. Atomic Energy Commn. U.C.R.L. 2417, 1953.

decision between the two types. This effect is dependent on symmetry and may be quite general for large conjugated loops. Analogous observations in other systems of known constitution tend to confirm the theoretical findings. Longuet-Higgins, Rector, and Platt [6] accounted for the bathochromic shift which accompanies hydrogenation of the porphin nucleus to the bacteriochlorophyll level by assuming structure (II) for the latter and used the simple M.O. method including overlap. We have extended the calculations to the $C_{2v}$ model, (III), as well as to chlorin, the dihydro-level. We find that the structure (III) is not associated with a bathochromic shift.

The $\pi$-electron system of porphin itself has the symmetry of the point group $D_{4h}$.* Like many conjugated systems with $D_{2h}$ or higher symmetries the low-lying $\pi$-electron transitions are to an unoccupied degenerate M.O. In consequence the spectrum predicted from simple M.O. theory might be expected to be altered, perhaps considerably, by configuration interaction.[7] Although Longuet-Higgins *et al.*[6] originally suggested that this alters the intensities of porphin rather than the predicted frequencies,* we have thought it better to compare the predicted spectra of the two tetrahydro-models with that of chlorin as none of these structures has degenerate levels and configuration interaction will be less serious than in porphin.

In applying M.O. methods to porphin molecules two rather serious approximations, besides those implicit in the general method, have to be made. These concern the value of $\alpha_N$, the Coulomb integral for nitrogen, and the variation of $\alpha_i$, the Coulomb integral of the $i$'th atom with its charge density $q_i$. It is convenient to express $\alpha_N$ in terms of $\alpha_C$ by means of equation (I) in which $\beta$ is the resonance integral for a carbon–carbon double bond :

$$\alpha_N = \alpha_C + \beta\delta \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

Values of $\delta$ ranging from 0·6 to 2·0 are found in the literature.[8] We have attempted to avoid the necessity of selecting one value for $\delta$ by examining an extreme range of values (*viz.*, 0—2·0). This is readily done by using the method of Coulson and Longuet-Higgins [9] in which the change in $\alpha$ is treated as a perturbation. $\delta\varepsilon_r$, the first order perturbation energy for the $r$'th M.O., is given by equation (2)

$$\delta\varepsilon_r = \sum c^2_{ir} \,.\, \delta\alpha_i \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where $c_{ir}$ is the coefficient of the atom $i$ in the $r$'th M.O. Longuet-Higgins *et al.*[6] have already applied this correction, with $\delta = 1$, to porphin and $D_{2h}$ tetrahydroporphin (II). We have also calculated sets of data in which $\alpha_N$ has been varied with $q$. The results for porphin, chlorin, and the two tetrahydroporphins have been collected in Table 1. Only the first transitions have been considered. The energy values have also been converted into wavelengths by use of conversion factors obtained by assuming the energy for the first transition of chlorin to correspond to 6375 Å, the experimentally determined position of the longest-wavelength band in chlorin, the structure of which has been rigidly proved.[10] The value of $\beta$ used to convert the energy into frequency is also included in Table 1 and may be compared with the values for $\beta$ of $-23{,}000$ and $-30{,}000$ cm.$^{-1}$ which best reproduce the experimental values in the polycyclic aromatic hydrocarbon series and the polyenes respectively.[7]

Fortunately we have certain empirical criteria which we may legitimately use to limit the choice of $\delta$ and its variation with charge density. If we select chlorin as a reference compound, the calculated data for the first transition must fulfil two conditions : (1) At least one of the two tetrahydro-structures (II) and (III) must have its first transition of lower energy than the first transition of chlorin. This follows from the fact that bacteriochlorophylls always have their longest-wavelength band some 1000 Å to longer wavelengths

* This symmetry is partially destroyed by the presence of the two pyrrolic hydrogen atoms but the assumption of $D_{4h}$ symmetry is a reasonable approximation.

[6] Longuet-Higgins, Rector, and Platt, *J. Chem. Phys.*, 1950, **18**, 1174.
[7] See Platt, *ibid.*, p. 1168, for a qualitative discussion of configuration interaction.
[8] Cf. Davies, *Trans. Faraday Soc.*, 1955, **51**, 449.
[9] Coulson and Longuet-Higgins, *Proc. Roy. Soc.*, 1947, *A*, **191**, 39.
[10] Eisner and Linstead, *J.*, 1955, 3742.

than that of the corresponding chlorins. (2) The first transition of a chlorin is always observed to be of lower energy than that of the corresponding porphin. The M.O. method with configuration interaction included should predict this sequence. But since configuration interaction tends to reduce the energies of first transitions and since its effect will be greater in porphin than in chlorin, it follows that simple M.O. theory without configuration interaction should predict the same sequence.

Two important conclusions may now be drawn from Table 1. First, the theory predicts that even for the extreme range of $\delta$ considered the tetrahydroporphin (II) of symmetry $D_{2h}$ always has its longest-wavelength band at longer wavelengths than that (III) of $C_{2v}$ symmetry. Secondly, if the choice of $\delta$ and its variation with $q$ is limited to those values which fulfil the two foregoing empirical criteria (*i.e.*, for values of $\delta$ between about 0·7 and 1·6), the predicted long-wavelength absorption of the $D_{2h}$ model (II) lies above that of chlorin, while that of the $C_{2v}$ compound lies below that of chlorin.

TABLE 1. *The energies, wavelengths, and symmetry types predicted for the longest-wavelength bands of porphin and its hydro-derivatives.*

| | $\delta$: | 0 | 0·6 * | | 0·8 | 1·0 * | | 1·2 | 1·4 * | | | 2·0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Porphin | $\Delta y/\beta$ | 0·391 | 0·543 | 0·491 | 0·435 | 0·588 | 0·627 | 0·583 | 0·534 | 0·665 | 0·641 | 0·662 | 0·620 | 0·529 |
| | $\lambda$ (Å) | 6300 | 5700 | 5300 | 4760 | 5590 | 5520 | 5180 | 4720 | 5476 | 5890 | 5070 | 4690 | 9040 |
| | | $A_{2g}-$ | | $A_{2u}-$ | | $A_{2u}-$ | | $A_{2u}-$ | | $A_{2u}-$ | $A_{1u}-$ | $A_{2u}-$ | $A_{2u}-$ | $A_{1u}-$ |
| | | $E_g$ | | $E_g$ | | $E_g$ | | $E_g$ | | $E_g$ | $E_g$ | $E_g$ | $E_g$ | $E_g$ |
| Chlorin | $\Delta y/\beta$ | 0·386 | 0·486 | 0·408 | 0·325 | 0·516 | 0·542 | 0·471 | 0·395 | 0·568 | 0·592 | 0·526 | 0·456 | 0·756 |
| | $\lambda$ (Å) | 6375 | 6375 | 6375 | 6375 | 6375 | 6375 | 6375 | 6375 | 6375 | 6375 | 6375 | 6375 | 6375 |
| | | $B_1-$ | | $B_1-$ | | $B_1-$ | | $B_1-$ | | $B_1-$ | | $B_1-$ | | $B_1-$ |
| | | $B_1$ | | $B_1$ | | $B_1$ | | $B_1$ | | $B_1$ | | $B_1$ | | $B_1$ |
| $D_{2h}$ Tetra-hydroporphin | $\Delta y/\beta$ | 0·380 | 0·514 | 0·446 | 0·383 | 0·497 | 0·449 | 0·468 | 0·464 | 0·402 | 0·356 | 0·374 | 0·393 | 0·225 |
| | $\lambda$ (Å) | 6480 | 6020 | 5840 | 5410 | 6620 | 7700 | 6380 | 5430 | 9010 | 10600 | 8970 | 7400 | 21200 |
| | | $B_{1u}-$ | | $B_{1u}-$ | | $A_u-$ | $A_u-$ | $A_u-$ | $B_{1u}-$ | $A_u-$ | | $A_u-$ | | $A_u-$ |
| | | $B_{3g}$ | | $B_{3g}$ | | $B_{3g}$ | $B_{3g}$ | $B_{3g}$ | $B_{3g}$ | $B_{3g}$ | | $B_{3g}$ | | $B_{3g}$ |
| $C_{2v}$ Tetra-hydroporphin | $\Delta y/\beta$ | 0·482 | 0·648 | 0·601 | 0·551 | 0·668 | 0·640 | 0·664 | 0·656 | 0·620 | 0·597 | 0·601 | 0·551 | 0·530 |
| | $\gamma$ (Å) | 5110 | 4780 | 4330 | 3760 | 4920 | 5410 | 4500 | 3840 | 5845 | 6320 | 5580 | 5280 | 9030 |
| | | $B_1-$ | | $B_1-$ | | $A_2-$ | $A_2-$ | $A_2-$ | $B_1-$ | $A_2-$ | $A_2-$ | $B_1-$ | $B_1-$ | $A_2-$ |
| | | $B_1$ | | $B_1$ | | $B_1$ | $B_1$ | $B_1$ | $B_1$ | $B_1$ | $B_1$ | $B_1$ | $B_1$ | $B_1$ |
| $-10^4\beta$ (cm.$^{-1}$) | | 4·06 | 3·23 | 3·84 | 4·83 | 3·04 | 2·89 | 3·35 | 3·97 | 2·76 | 2·65 | 2·98 | 3·44 | 2·09 |

* The first set refers to $\delta$; the second to $(\delta + 1 - q)$, and the third to $(\delta + 2 - 2q)$.

It is fortunate that there exists one series in which all four compounds and their spectra are probably known, *viz.*, the *meso*tetraphenyl series. Dorough and Miller [11] have prepared the two tetrahydro-pigments and have established their higher state of hydrogenation by dehydrogenation to the chlorin.* Results for the longest-wavelength band of these compounds are given in Table 2, together with similar values for other incomplete series.

TABLE 2. *Wavelengths* (Å) *of the longest-wavelength bands of some porphin derivatives.*

*meso*Tetraphenylporphin,[a] **6480.**
*meso*Tetraphenylchlorin,[a,b] **6550.**
Tetrahydrotetraphenylporphin compound **α**,[b] 7500.
Tetrahydrotetraphenylporphin compound **β**,[b] 5910.
Porphin,[c] 6160.
Chlorin,[c] 6375.
Oxophæoporphin-$a_5$ monomethyl ester, **6455.**
2-Acetyl-2-devinylmethylphæophorbide **a**,[d] **6850.**
Bacteriophæophytin,[f] 7500.

[a] Ball, Dorough, and Calvin, *J. Amer. Chem. Soc.*, 1946, **68**, 2278; [b] Dorough and Miller, *ibid.*, 1952, **74**, 6106; [c] Eisner and Linstead, *J.*, 1955, 3742; [d] Fischer, Lautsch, and Lin, *Annalen*, 1938, **534**, 1; [e] Fischer and Riedmair, *ibid.*, 1933, **505**, 87; [f] Weigl, *J. Amer. Chem. Soc.*, 1953, **75**, 999.

It is seen in the *meso*tetraphenyl series that the two tetrahydro-pigments are in fact in the predicted positions relative to the chlorin. The remarkable spectral difference predicted

* The proof that these compounds are actually at the tetrahydro- rather than the hexahydro-level is still required, although Seely [8] has some evidence for the separate existence of the hexahydro-derivative which, if correct, locates the longest-wavelength band at 6420 Å for the zinc derivative.

[11] Dorough and Miller, *J. Amer. Chem. Soc.*, 1952, **74**, 6106.

for the two types of symmetry is therefore experimentally established and we can with confidence assign structure (I) to bacteriochlorophyll.

Our results seem quite general for the symmetry changes involved, for the predictions based on calculations in the porphin series may be successfully applied to the polycyclic aromatic hydrocarbon series. Longuet-Higgins *et al.*[6] have already noted the parallelism between the series porphin, chlorin, and $D_{2h}$ tetrahydroporphin, and the series coronene, 1 : 2-benzoperylene, and perylene. We now find that $C_{2v}$ tetrahydroporphin and 1 : 2-benzopyrene may be included in the respective series (cf. Table 3).

TABLE 3. *Comparison of the longest-wavelength* (Å) *bands of* meso-*tetraphenylporphins and polycyclic aromatic hydrocarbons.**

| $D_{4h}$ | $C_{2v}$ | $D_{2h}$ | $C_{2v}$ |
|---|---|---|---|
| | | | |
| 3415 | 3875 | 4340 | 3315 |
| 3360 | 3670 | 4060 | 3165 |
| 3255 | 3480 | 3870 | 3040 |
| 3195 | 3310 | 3860 | |
| 3165 | | | |
| $D_{4h}$ | $C_{2v}$ | $D_{2h}$ | $C_{2v}$ |
| Porphin | Chlorin | Tetrahydro | Tetrahydro |
| 6480 | 6550 | 7500 | 5910 |

* The data for the hydrocarbons refer to the *p*-bands (Clar, " Aromatische Kohlenwasserstoffe," Springer, Berlin, 1952.

The results described here provide an example of the manner in which the methods of theoretical chemistry, in spite of their many approximations, can be employed for solving structural problems. We have purposely avoided the choice of a single value for $\delta$. It is gratifying to note, however, that the experimental results are best reproduced by $\delta \sim 1$ which is the value generally assumed for this parameter.[12] Further the values of $\beta$ in this region agree satisfactorily with the value usually assumed (see p. 1174). If the value of $\delta$ is approximately unity, the 7500 Å band of $D_{2h}$ tetrahydroporphin is due to the $A_g$–$B_{3g}$ transition and is polarised parallel to the long axis. The method of correction for the variation of $\alpha$ with $q_i$ is, at best, a very crude approximation. We have used the $q_i$'s derived for the all-carbon model and have only considered the variation of $\delta$ for the four nitrogen atoms. Although the errors involved cannot alter the present conclusions a more satisfactory treatment is necessary for the predictions of other properties of these molecules.

## CALCULATIONS

We have corrected all M.O. energies for overlap by the usual method [13] in which

$$y_r = 4x_r/(4 + x_r)$$

where

$$x_r = (\varepsilon_r - \alpha)/\beta$$

$\varepsilon_r$ being the enery of the $r$'th M.O. This is an approximation to the more accurate correction used by Wheland [14] when the coulomb integrals vary.

As no account of spin interaction is included the predicted transition energies refer to the centres of gravity of the singlet–singlet and singlet–triplet transitions. We have made no explicit correction for the effect of the two pyrrolic hydrogen atoms. Their effect is probably

[12] Brown, *Quart. Rev.*, 1952, **6**, 63.
[13] Wheland, *J. Amer. Chem. Soc.*, 1941, **63**, 2025.
[14] *Idem, ibid.*, 1942, **64**, 900.

small and acts so as to reduce $\alpha_N$. It is therefore absorbed by the range of values considered for the variation of $\delta_i$ with $q_i$.

*Porphin and* $D_{2h}$ *Tetrahydroporphin.*—The M.O. energies and $c_i$'s have been calculated by Longuet-Higgins, Rector, and Platt.[6] We have used these values to calculate the additional data for the various $\delta$'s.

*Chlorin.*—A recent theoretical treatment of this compound gives secular equations which include explicit corrections for the pyrrolic hydrogen atoms but take no account of the variation of $\alpha_i$ with $q_i$.[15] Chlorin belongs to the symmetry group $C_{2v}$, the M.O. wave functions being bases of the irreducible representations $B_1$ and $A_2$. The roots of the secular equations and the corresponding coefficients are given in Table 4. Table 5 lists the charge densities at the various positions.

TABLE 4. *Values of* $x_r$ *and* $c_{ir}$ *for chlorin.*

*Representation* $A_2$.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| $x_r$ | −2·0655 | −1·9197 | −1·6180 | −0·9247 | −0·4680 | 0·4472 | 0·6180 | 1·0985 | 1·5805 | 2·2517 |
| $c_1$ | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 |
| $c_2$ | 0·0605 | 0·1491 | 0·0000 | 0·4176 | 0·1700 | 0·3851 | 0·0000 | 0·2844 | 0·1885 | 0·0782 |
| $c_3$ | −0·1250 | −0·2863 | 0·0000 | −0·3862 | −0·0796 | 0·1722 | 0·0000 | 0·3124 | 0·2979 | 0·1760 |
| $c_4$ | 0·1976 | 0·4005 | 0·0000 | −0·0605 | −0·1328 | −0·3081 | 0·0000 | 0·0588 | 0·2824 | 0·3181 |
| $c_5$ | −0·0174 | −0·3175 | −0·1730 | 0·2734 | −0·1030 | −0·0897 | −0·3897 | −0·2042 | 0·1294 | 0·2554 |
| $c_6$ | −0·1618 | 0·2091 | 0·2799 | −0·1923 | 0·1810 | 0·2679 | −0·2408 | −0·2830 | −0·0778 | 0·2570 |
| $c_7$ | 0·3515 | −0·0839 | −0·2799 | −0·0956 | 0·0183 | 0·2096 | 0·2409 | −0·1067 | −0·2524 | 0·3233 |
| $c_8$ | −0·2984 | 0·1168 | 0·0000 | 0·1119 | −0·4342 | 0·0461 | 0·0000 | 0·2095 | −0·3401 | 0·1862 |
| $c_9$ | 0·2648 | −0·1404 | 0·2799 | −0·0079 | 0·1850 | −0·1890 | −0·2409 | 0·3368 | −0·2851 | 0·0958 |
| $c_{10}$ | −0·2485 | 0·1527 | −0·4529 | −0·1046 | 0·3476 | −0·1306 | −0·1489 | 0·1605 | −0·1105 | 0·0295 |
| $c_{20}$ | −0·2659 | −0·1649 | 0·1730 | 0·1688 | 0·2447 | −0·2203 | 0·3897 | −0·0437 | 0·0190 | 0·2849 |
| $c_{21}$ | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 |

*Representation* $B_1$.

| | | | | | | |
|---|---|---|---|---|---|---|
| $x_r$ | −3·1447 | −2·0306 | −1·6180 | −1·5167 | −0·3519 | 0·0000 |
| $c_1$ | 0·1278 | 0·2877 | 0·0000 | 0·5074 | 0·3327 | 0·3780 |
| $c_2$ | −0·1370 | −0·2921 | 0·0000 | −0·3848 | −0·0586 | 0·0000 |
| $c_3$ | 0·1661 | 0·3054 | 0·0000 | 0·0762 | −0·3122 | −0·3780 |
| $c_4$ | −0·2192 | −0·3281 | 0·0000 | 0·2692 | 0·1684 | 0·0000 |
| $c_5$ | 0·0256 | 0·2395 | −0·2021 | −0·2648 | 0·2578 | 0·0000 |
| $c_6$ | 0·1643 | −0·1582 | 0·3271 | 0·1325 | −0·2591 | 0·0000 |
| $c_7$ | −0·3268 | 0·0818 | −0·3271 | 0·0639 | −0·1666 | 0·0000 |
| $c_8$ | 0·2820 | −0·1291 | 0·0000 | −0·0098 | 0·3226 | −0·3780 |
| $c_9$ | −0·2780 | 0·1805 | 0·3271 | −0·0491 | 0·0531 | 0·0000 |
| $c_{10}$ | 0·0884 | −0·0596 | −0·1249 | 0·0195 | −0·0393 | 0·0000 |
| $c_{20}$ | 0·2546 | 0·1213 | 0·2021 | −0·2196 | −0·0049 | 0·3780 |
| $c_{21}$ | 0·2593 | −0·1777 | −0·4043 | 0·0647 | −0·3020 | 0·0000 |
| $x_r$ | 0·6180 | 0·7183 | 1·0965 | 1·7365 | 2·1314 | 2·3612 |
| $c_1$ | 0·0000 | 0·1995 | 0·3315 | 0·4795 | 0·1695 | 0·0683 |
| $c_2$ | 0·0000 | 0·0717 | 0·1818 | 0·4163 | 0·1806 | 0·0806 |
| $c_3$ | 0·0000 | −0·1481 | −0·1322 | 0·2434 | 0·2155 | 0·1221 |
| $c_4$ | 0·0000 | −0·1780 | −0·3267 | 0·0664 | 0·2786 | 0·2077 |
| $c_5$ | −0·2538 | 0·2760 | −0·2151 | −0·1063 | 0·1977 | 0·1661 |
| $c_6$ | −0·1568 | 0·3763 | 0·0909 | −0·1909 | 0·1427 | 0·1846 |
| $c_7$ | 0·1568 | −0·0057 | 0·3148 | −0·2253 | 0·1065 | 0·2697 |
| $c_8$ | 0·0000 | −0·1246 | 0·2652 | −0·0743 | −0·0964 | 0·2501 |
| $c_9$ | −0·1568 | −0·0838 | −0·0240 | 0·0963 | −0·3120 | 0·3208 |
| $c_{10}$ | 0·4106 | 0·2973 | −0·2490 | 0·1308 | −0·2758 | 0·2356 |
| $c_{20}$ | 0·2538 | −0·2558 | −0·0109 | −0·1260 | 0·1807 | 0·2022 |
| $c_{21}$ | −0·5076 | −0·2332 | −0·0438 | 0·1110 | −0·2928 | 0·2717 |

TABLE 5. *Charge densities in chlorin.*

| Position | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $q_i$ ..... | 1·1117 | 1·0428 | 1·0996 | 1·0771 | 1·0968 | 1·0947 | 1·0802 | 1·0046 | 1·0786 | 1·0915 | 1·2921 | 0·9715 |

$C_{2v}$ *Tetrahydroporphin.*—As in chlorin the M.O. wave functions are bases of the irreducible representations $A_2$ and $B_1$. A further simplification can be made as the $A_2$ wave functions are bases of the irreducible representations $A_2'$ and $B_1'$ of the group corresponding to $A_2$ which is of

[15] Matlow, *J. Chem. Phys.*, 1955, **23**, 673.

course isomorphous with $C_{2v}$. In this way the ninth-order secular determinant of $A_2$ factorises seventh- and second-order determinants. Table 6 lists the roots of the secular equations and the corresponding coefficients. The charge densities are given in Table 7.

TABLE 6. *Values of $x_r$ and $c_{ir}$ for $C_{2v}$ tetrahydroporphin.*

| | $A_2'$ | | $B_1'$ | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Representation $A_2$. | | | | | | | |
| $x_r$ ..... | −1·6180 | 0·6180 | −2·0000 | −1·4812 | −0·6180 | 0·3111 | 1·0000 | 1·6180 | 2·1701 |
| $c_1$ ..... | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 |
| $c_2$ ..... | 0·0000 | 0·0000 | 0·0816 | 0·2779 | 0·3804 | 0·3621 | 0·2887 | 0·2351 | 0·0408 |
| $c_3$ ..... | 0·0000 | 0·0000 | −0·1633 | −0·4117 | −0·2351 | 0·1126 | 0·2887 | 0·3804 | 0·0886 |
| $c_4$ ..... | 0·0000 | 0·0000 | 0·2449 | 0·3318 | −0·2351 | −0·3270 | 0·0000 | 0·3804 | 0·1515 |
| $c_5$ ..... | 0·0000 | 0·0000 | −0·3266 | −0·0798 | 0·3804 | −0·2144 | −0·2887 | 0·2351 | 0·2401 |
| $c_6$ ..... | 0·0000 | 0·0000 | 0·4082 | −0·2136 | 0·0000 | 0·2603 | −0·2887 | 0·0000 | 0·3696 |
| $c_7$ ..... | 0·2629 | 0·4254 | −0·2449 | 0·1981 | −0·1902 | 0·1477 | 0·0000 | −0·1176 | 0·2810 |
| $c_8$ ..... | 0·4254 | 0·2629 | 0·0816 | −0·0798 | 0·1176 | −0·2144 | 0·2887 | −0·1902 | 0·2401 |
| $c_9$ ..... | −0·4254 | −0·2629 | 0·0816 | −0·0798 | 0·1176 | −0·2144 | 0·2887 | −0·1902 | 0·2401 |
| $c_{10}$ ... | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 | 0·0000 |
| $c_{19}$ ... | −0·2629 | −0·4254 | −0·2449 | 0·1981 | −0·1902 | 0·1477 | 0·0000 | −0·1176 | 0·2810 |

| | Representation $B_1$. | | | | | |
|---|---|---|---|---|---|---|
| $x_r$ ..... | −2·1242 | −2·0000 | −1·7855 | −1·0977 | −0·4303 | 0·0000 |
| $c_1$ ..... | 0·0801 | 0·2357 | 0·4148 | 0·3974 | 0·2243 | 0·3536 |
| $c_2$ ..... | −0·0851 | −0·2357 | −0·3703 | −0·2181 | −0·0483 | 0·0000 |
| $c_3$ ..... | 0·1007 | 0·2357 | 0·2464 | −0·1580 | −0·2035 | −0·3536 |
| $c_4$ ..... | −0·1287 | −0·2357 | −0·0696 | 0·3916 | 0·1361 | 0·0000 |
| $c_5$ ..... | 0·1727 | 0·2357 | −0·1221 | −0·2718 | 0·1450 | 0·3536 |
| $c_6$ ..... | −0·2382 | −0·2357 | 0·2876 | −0·0932 | −0·1983 | 0·0000 |
| $c_7$ ..... | 0·0261 | 0·2357 | −0·2539 | 0·2410 | −0·2484 | 0·0000 |
| $c_8$ ..... | 0·1828 | −0·2357 | 0·1658 | −0·1714 | 0·3052 | 0·0000 |
| $c_9$ ..... | −0·4143 | 0·2357 | −0·0421 | −0·0529 | 0·1171 | 0·0000 |
| $c_{10}$ ... | 0·3901 | −0·2357 | 0·0472 | 0·0964 | 0·5443 | 0·3536 |
| $c_{19}$ ... | 0·3072 | 0·0000 | −0·1375 | 0·1331 | 0·1886 | −0·3536 |
| $x_r$ ..... | 0·6495 | 1·0000 | 1·5345 | 1·9191 | 2·3346 | |
| $c_1$ ..... | 0·2187 | 0·3333 | 0·3255 | 0·4082 | 0·0333 | |
| $c_2$ ..... | 0·0710 | 0·1667 | 0·2498 | 0·3916 | 0·0389 | |
| $c_3$ ..... | −0·1725 | −0·1667 | 0·0577 | 0·3435 | 0·0575 | |
| $c_4$ ..... | −0·1831 | −0·3333 | −0·1612 | 0·2675 | 0·0953 | |
| $c_5$ ..... | 0·0563 | −0·1667 | −0·3050 | 0·1699 | 0·1649 | |
| $c_6$ ..... | 0·2179 | 0·1667 | −0·3069 | 0·0585 | 0·2898 | |
| $c_7$ ..... | −0·2703 | 0·3333 | −0·1449 | −0·0124 | 0·2331 | |
| $c_8$ ..... | −0·3935 | 0·1667 | 0·0846 | −0·0822 | 0·2543 | |
| $c_9$ ..... | 0·0148 | −0·1667 | 0·2747 | −0·1453 | 0·3606 | |
| $c_{10}$ ... | 0·0454 | −0·3333 | 0·3580 | −0·1515 | 0·3089 | |
| $c_{19}$ ... | 0·3582 | 0·0000 | −0·0210 | −0·0453 | 0·2786 | |

TABLE 7. *Charge densities in $C_{2v}$ tetrahydroporphin.*

| Position ... | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $q_i$ ..... | 1·1153 | 1·0429 | 1·1115 | 1·0517 | 1·0946 | 1·0892 | 1·1049 | 1·1068 | 1·0937 | 0·9694 | 1·2578 |

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## 246. *Modified Steroid Hormones. Part II.** (i) *Monohalogenation of 5α : 6β-Dibromocholestan-3-one.* (ii) *Some 2α-Halogenated Androgens.*

By Bernard Ellis and Vladimir Petrow.

A method has been developed for the preparation of 2α-halogenated cholest-4-en-3-ones (VIII*a*) from 5α : 6β-dibromocholestan-3-one (IV*a*), and extended to the preparation of some 2α-halogenated androgens.

Some 2-halogenated derivatives (VIII*b* and VIII*c*) of testosterone and methyltestosterone were required for biological study. Their preparation by the method used for 2-bromotestosterone hexahydrobenzoate [1] was unattractive as both 3-hydroxyandrostanone and 3-hydroxymethylandrostanone are relatively inaccessible. Halogenation at position 2 of the corresponding 5α : 6β-dibromo-17β-hydroxyandrostan-3-ones, followed by 5 : 6-debromination, seemed a more attractive route. Barton and Miller,[2] it is true, had stated that such halogenation at this position was improbable on mechanistic grounds. The work of Djerassi *et al.*,[3] however, appeared to indicate the feasibility of this approach and we therefore re-examined the bromination of 5α : 6β-dibromocholestan-3-one (IV*a*). The results obtained with this compound confirmed our expectations regarding the halogenation (section i) so we also studied the preparation of the required 2-halogenated androgens (section ii).

(i) By monobrominating 5α : 6β-dibromocholestan-3-one [4] (IV*a*) in ether–acetic acid, Inhoffen [5] obtained a tribromo-ketone, m. p. 138°. A second tribromo-ketone, m. p. 106°, was obtained almost simultaneously by Butenandt and Schramm,[6] who carried out the monobromination in acetic acid alone. The two compounds were regarded by their discoverers as epimeric 4 : 5 : 6-tribromocholestan-3-ones [6] (see also Corey [7]), not only on account of their chemical reactions, but also because of their conversion into the same tetrabromo-ketone, formulated as a 4 : 4 : 5 : 6-tetrabromocholestan-3-one. The higher-melting isomer, on treatment with potassium acetate, was shown to lose the elements of hydrogen bromide to give an unsaturated ketone, regarded as a 4 : 6-dibromocholest-4-en-3-one [5b] and additionally obtained [5c] from cholestenone and 6-bromocholestenone by di- and mono-bromination, respectively.

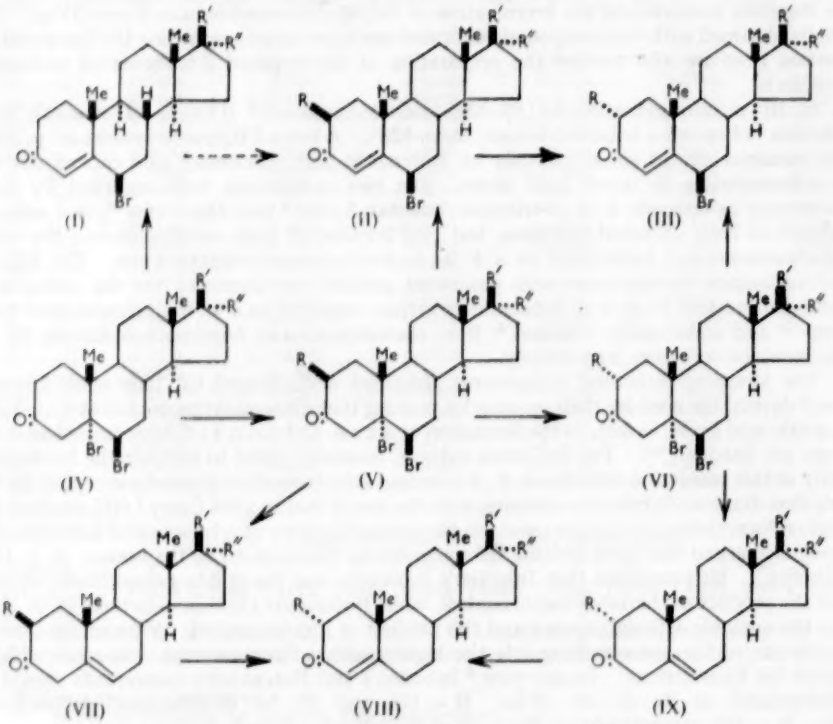
The foregoing structural assignments remained unchallenged till 1950 when Djerassi *et al.*[3] showed the need for their revision by proving that dibromination of cholest-4-en-3-one in acetic acid leads, in fact, to the formation of a 2 : 6- and not a 4 : 6-dibromocholest-4-en-3-one (cf. Inhoffen [5c]). The American authors, however, failed to indicate the incompatibility of this conclusion with the 4 : 5 : 6-tribromo-structures then generally accepted for the Inhoffen–Butenandt tribromo-ketones, with the result that in 1954 Corey [7] still retained the original formulations in his discussion on the stereochemistry of α-brominated keto-steroids. Corey isomerised the lower-melting tribromo-ketone (Butenandt) to the isomer, m. p. 138° (Inhoffen). He concluded that Inhoffen's substance was the stable 4α(equatorial)-epimer and the product of thermodynamic control, while Butenandt's tribromo-ketone, m. p. 106°, was the unstable 4β(axial)-epimer and the product of kinetic control. We confirm Corey's conversion of the lower-melting into the higher-melting bromo-ketone, but are unable to accept his formulations. In our view [8] Inhoffen's and Butenandt's compounds should be reformulated as 2α : 5α : 6β- (VI*a*; R = Br) and 2β : 5α : 6β-tribromocholestan-3-one (V*a*; R = Br), respectively, on the basis of the following transformations.

* Part I, *J.*, 1956, 627.

[1] Inhoffen and Zuhlsdorff, *Ber.*, 1943, **76**, 233; Djerassi and Scholz, *J. Amer. Chem. Soc.*, 1947, **69**, 2404.
[2] Barton and Miller, *ibid.*, 1950, **72**, 1066.
[3] Djerassi, Rosenkranz, Romo, Kaufmann, and Pataki, *ibid.*, p. 4534.
[4] See ref. 2 for the stereochemistry of the compound.
[5] Inhoffen, *Ber.*, 1936, **69**, (*a*) 1134, (*b*) 1702, (*c*) 2141.
[6] Butenandt and Schramm, *ibid.*, p. 2289.
[7] Corey, *J. Amer. Chem. Soc.*, 1954, **76**, 175.
[8] Cf. Fieser, Romero, and Fieser, *ibid.*, 1955, **77**, 3305, which appeared while our work was in manuscript.

Partial dehydrobromination of the Butenandt tribromo-ketone (V*a*; R = Br) by brief warming with pyridine gave 2β : 6β-dibromocholest-4-en-3-one (II*a*; R = Br), which passed smoothly in the presence of mineral acid into the more stable 2α-epimer (III*a*; R = Br), also obtained directly from the Inhoffen tribromo-ketone (VI*a*; R = Br) by similar treatment with pyridine. The β-configuration is assigned to the 6-bromine atom in both (II*a*; R = Br) and (III*a*; R = Br) as (*a*) partial dehydrobromination of the dibromo-compound (IV*a*) under similar conditions leads to the formation of 6β- (I*a*; R = Br) and not 6α-bromocholest-4-en-3-one [2] and (*b*) treatment of the monobromo-compound (I*a*) with mineral acids under conditions effecting the change of the 2β- (II*a*) into the 2α-compound (III*a*) leads only to recovery of starting material.

The ultraviolet absorption spectrum of 2β : 6β-dibromocholest-4-en-3-one (II*a*; R = Br) shows a maximum at 257 mμ in contrast to that at 248—250 mμ reported [2c,3] for the 2-epimeric dibromo-ketone (III*a*; R = Br). There is, consequently, a bathochromic shift in passing from the 2α(equatorial)- to the 2β(axial)-bromo-substituent. A bathochromic shift of similar order has also been observed with certain epimeric 6-bromo-3-oxo-$\Delta^4$-steroids.[2,3]




(*a*) R′ = $C_8H_{17}$, R″ = H. (*b*) R′ = OAc, R″ = H. (*c*) R′ = OH, R″ = Me.

Partial debromination of the tribromo-ketones (V*a* and VI*a*; R = Br) by brief treatment with sodium iodide in acetone furnished two epimeric unsaturated bromo-ketones which lacked significant ultraviolet absorption, and to which the constitutions 2β- (VII*a*; R = Br) and 2α-bromocholest-5-en-3-one (IX*a*; R = Br), respectively, have been assigned. Both compounds were readily isomerised to 2α-bromocholest-4-en-3-one (VIII*a*; R = Br), a known ketone of proved structure.[9]

[9] Djerassi, *J. Amer. Chem. Soc.*, 1949, **71**, 1003.

Monochlorination of the dibromo-ketone (IV*a*) in acetic acid gave 5α : 6β-dibromo-2β-chlorocholestan-3-one (V*a*; R = Cl), which could be isolated only when the mixture was worked up immediately absorption of chlorine was complete; otherwise epimerisation occurred, to give the 2α-isomer (VI*a*; R = Cl). Partial dehydrobromination of these chlorinated derivatives with warm pyridine furnished the epimeric unsaturated chloro-bromo-ketones (II*a* and III*a*; R = Cl), respectively. The latter compound was also obtained by epimerisation of the former with mineral acid, and by monochlorination of 6β-bromocholest-4-en-3-one (I). The ultraviolet absorption spectrum of the 2α-compound (III*a*; R = Cl) showed a maximum at 249 mμ which corresponds closely to that of its 2α-bromo-analogue (III*a*; R = Br). The isomeric chloro-bromo-ketone gave a maximum at 254 mμ, a value consistent with the proposed formulation (II*a*; R = Cl).

2β- (VII*a*; R = Cl) and 2α-Chlorocholest-5-en-3-one (IX*a*; R = Cl), obtained from the chlorodibromo-compounds (V*a* and VI*a*; R = Cl), respectively, by debromination with sodium iodide, were severally isomerised by mineral acid to 2α-chlorocholest-4-en-3-one (VIII*a*; R = Cl), identical with a specimen prepared by an alternative route.[19]

(ii) Bromination of 17α-methylandrost-5-ene-3β : 17β-diol, followed by oxidation with chromium trioxide, furnished 5α : 6β-dibromo-17β-hydroxy-17α-methylandrostan-3-one (IV*c*). Monochlorination of the latter in acetic acid gave the corresponding 2β- (V*c*; R = Cl) and 2α-chloro-derivative (VI*c*; R = Cl), depending upon the conditions employed. Debromination of the 2β-isomer with sodium iodide followed by isomerisation of the product by mineral acid afforded the required 2α-chloro-17α-methyltestosterone (VIII*c*; R = Cl).

2α-Chlorotestosterone acetate (VIII*b*; R = Cl) was prepared by the reaction sequence: 17β-acetoxyandrost-5-en-3β-ol ⟶ 5α : 6β-dibromide ⟶ 3-ketone ⟶ 2β-chloro-derivative followed by debromination and isomerisation. 2α-Bromomethyltestosterone (VIII*c*; R = Br) was similarly obtained.

## Experimental

Optical rotations were measured for $CHCl_3$ solutions in a 1 dm tube. Ultraviolet absorption spectra (in propan-2-ol) were kindly determined by Mr. M. Davies, B.Sc. (values in parentheses are log ε).

2β : 6β-*Dibromocholest-4-en-3-one* (II; R = Br).—2β : 5α : 6β-Tribromocholestan-3-one (m. p. 106°, $[\alpha]_D^{21}$ +9°) (1 g.) in dry pyridine (5 ml.) was warmed for 3 min. on the steam-bath. 2β : 6β-*Dibromocholest-4-en-3-one* crystallised from acetone–methanol as prisms (0·6 g.), m. p. 124° (decomp.), $[\alpha]_D^{20}$ −41° (*c*, 0·54), $\lambda_{max.}$ 257 mμ (4·08) (Found: C, 59·5; H, 7·5. $C_{27}H_{42}OBr_2$ requires C, 59·8; H, 7·8%).

2α : 6β-*Dibromocholest-4-en-3-one* (III; R = Br).—(*a*) The foregoing compound (200 mg.) in ether (10 ml.) and acetic acid (10 ml.) was treated with 10 drops of hydrogen bromide in acetic acid (50% w/w) and kept for 18 hr. at room temperature. Crystallisation of the product from acetone–methanol gave 2α : 6β-dibromocholest-4-en-3-one (150 mg.), needles, m. p. 162—163°, not depressed on admixture with a specimen prepared by dibrominating cholest-4-en-3-one.

(*b*) Treatment of 2α : 5α : 6β-tribromocholestan-3-one (m. p. 138°, $[\alpha]_D^{21}$ −44·5°) (1·5 g.) with pyridine (8 ml.) for 3 min. at 100° gave a product (1 g.) which crystallised from acetone–methanol in needles, m. p. 162—163°, not depressed on admixture with a specimen prepared by method (*a*).

6β-*Bromocholest-4-en-3-one* (I).—5α : 6β-Dibromocholestan-3-one (5 g.) in pyridine (20 ml.) was warmed for 2 min. on the steam-bath, and the solids obtained on dilution with water were purified from aqueous ethanol, to give needles (3·3 g.) of 6β-bromocholest-4-en-3-one, m. p. and mixed m. p. 130—132°. The compound was recovered unchanged after treatment with hydrogen bromide in ether–acetic acid.

2β-*Bromocholest-5-en-3-one* (VII; R = Br).—Sodium iodide (4 g.) was added to 2β : 5α : 6β-tribromocholestan-3-one (3·6 g.) in acetone (60 ml.). The mixture was stirred for 5 min., then diluted with 5% aqueous sodium thiosulphate, precipitating an oil which soon solidified. Purified from aqueous acetone, 2β-*bromocholest-5-en-3-one* formed needles (1·7 g.), m. p. 109—110° (decomp.), $[\alpha]_D^{20}$ +115° (*c*, 1·12) (Found: C, 70·1; H, 9·3. $C_{27}H_{43}OBr$ requires C, 69·95; H, 9·35%).

[19] Ellis and Petrow, *J.*, 1953, 3869; Beereboom, Djerassi, Ginsberg, and Fieser, *J. Amer. Chem. Soc.*, 1953, **75**, 3500.

*2α-Bromocholest-5-en-3-one* (IX; R = Br), prepared from 2α : 5α : 6β-tribromocholestan-3-one (1·4 g.) by the foregoing procedure, separated (0·75 g.) from aqueous acetone in needles, m. p. 109—110°, $[\alpha]_D^{19}$ +0·5° (*c*, 1·02) (Found : C, 70·0; H, 9·4. $C_{27}H_{43}OBr$ requires C, 69·95; H, 9·35%).

*2α-Bromocholest-4-en-3-one* (VIII; R = Br).—The foregoing compound (500 mg.) in ether (25 ml.) and acetic acid (12·5 ml.) was treated with concentrated hydrochloric acid (0·5 ml.). Next morning, the mixture was poured into water and the product isolated with ether. 2α-Bromocholest-4-en-3-one (350 mg.) formed needles (from methanol), m. p. 132—134°, $[\alpha]_D^{19}$ +94° (*c*, 0·8), $\lambda_{max.}$ 245 mμ (4·13) (Found : C, 69·8; H, 9·25%) {Djerassi [9] gives m. p. 117—119°, $[\alpha]_D^{20}$ +81°, $\lambda_{max.}$ 243 mμ (4·15).} Reaction with 2 : 4-dinitrophenylhydrazine in hot acetic acid gave the deep red cholesta-4 : 6-dien-3-one 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 227—228°.

The compound (VIII; R = Br) was also obtained by similar isomerisation of (VII; R = Br).

*Monochlorination of 5α : 6β-Dibromocholestan-3-one.*—Chlorine (287 mg., 1 mol.) was added to 5α : 6β-dibromocholestan-3-one (2·2 g.) in acetic acid (200 ml.). When absorption of chlorine was complete (*ca.* 30 min.), the mixture was poured into water (200 ml.) and the solids were collected, washed, and air-dried. Purified from acetone–methanol, *5α : 6β-dibromo-2β-chlorocholestan-3-one* (1·6 g.) formed prisms, m. p. 114—116° (decomp.), $[\alpha]_D^{20}$ +2° (*c*, 1·1) (Found : C, 55·9; H, 7·4. $C_{27}H_{43}OClBr_2$ requires C, 56·0; H, 7·5%). The epimeric *5α : 6β-dibromo-2α-chlorocholestan-3-one* (1·5 g.), needles (from acetone–methanol), m. p. 150—151° (decomp.), $[\alpha]_D^{21}$ −41° (*c*, 1·29) (Found : C, 55·5; H, 7·3%), was obtained when the mixture was set aside overnight.

*6β-Bromo-2β-chlorocholest-4-en-3-one* (II; R = Cl).—5α : 6β-Dibromo-2β-chlorocholestan-3-one (5 g.) in pyridine (20 ml.) was warmed for 3 min. on the steam-bath. The solids obtained by the addition of water crystallised from acetone–methanol, giving the bromochloro-ketone (3·4 g.), m. p. 110—112°, $[\alpha]_D^{21}$ −39° (*c*, 0·96), $\lambda_{max.}$ 254 mμ (4·09) (Found : C, 64·5; H, 8·4. $C_{27}H_{43}OClBr$ requires C, 65·1; H, 8·5%).

*6β-Bromo-2α-chlorocholest-4-en-3-one* (III; R = Cl).—(*a*) The foregoing compound (500 mg.) in ether (25 ml.) and acetic acid (15 ml.) was treated with concentrated hydrochloric acid (0·5 ml.). After 18 hr. at 0°, the mixture was poured into water, and the product isolated with ether. The *bromochloro-ketone* (400 mg.) crystallised from acetone–methanol in needles, m. p. 144—145° (decomp.), $[\alpha]_D^{22}$ +39° (*c*, 1·09), $\lambda_{max.}$ 249 mμ (4·09) (Found : C, 65·3; H, 8·5%).

(*b*) Partial dehydrobromination of 5α : 6β-dibromo-2α-chlorocholestan-3-one (1 g.) with warm pyridine (5 ml.) gave 6β-bromo-2α-chlorocholest-4-en-3-one (600 mg.), needles (from acetone–methanol), m. p. 145° (decomp.), identical with the compound prepared by method (*a*).

(*c*) A stirred solution of 6β-bromocholest-4-en-3-one (4·1 g.) in ether (100 ml.) at 0° was treated with chlorine (0·63 g., 1 mol.) in acetic acid (20 ml.) dropwise during 15 min. After 24 hr. at 0°, the product was isolated and purified from acetone–methanol, to give the bromochloro-ketone (2·5 g.), m. p. and mixed m. p. 144—145°.

*2β-Chlorocholest-5-en-3-one* (VII; R = Cl).—5α : 6β-Dibromo-2β-chlorocholestan-3-one (5·4 g.) in acetone (60 ml.) at 35° was treated with sodium iodide (7 g.). The mixture was stirred for 5 min., then poured into water, and the product extracted with ether. The extract was washed with aqueous sodium thiosulphate and water, then dried, and the solvent removed *in vacuo*. The insoluble fraction obtained on trituration of the residue with a small volume of cold ethanol was purified from aqueous ethanol, to give the chloro-ketone (2·6 g.), needles, m. p. 114—116°, $[\alpha]_D^{21}$ +98° (*c*, 0·59) (Found : C, 76·8; H, 10·3. $C_{27}H_{43}OCl$ requires C, 77·4; H, 10·35%).

*2α-Chlorocholest-5-en-3-one* (IX; R = Cl), prepared from the 2α-compound (VI; R = Cl) (3 g.) by the foregoing procedure, separated (1·7 g.) from aqueous acetone in needles or plates, m. p. 141—142°, $[\alpha]_D^{22}$ −5° (*c*, 1·09) (Found : C, 76·9; H, 10·1%).

*2α-Chlorocholest-4-en-3-one* (VIII; R = Cl).—The foregoing compound (1 g.) in ether (50 ml.) and acetic acid (30 ml.) was treated with concentrated hydrochloric acid (1 ml.), then kept overnight at room temperature. The product crystallised from methanol, 2α-chlorocholest-4-en-3-one (0·9 g.) separating in needles, $[\alpha]_D^{21}$ +86°, m. p. 98°, alone or mixed with an authentic specimen.[10]

*5α : 6β-Dibromo-17β-hydroxy-17α-methylandrostan-3-one* (IV*c*).—17α-Methylandrost-5-ene-3β : 17β-diol (12 g.) in acetic acid (320 ml.) was treated with bromine (6·4 g.) in acetic acid (40 ml.). After the addition of chromium trioxide (4 g.) in acetic acid (40 ml. of 80%), the mixture was kept for 2 hr., then poured into water. The washed and air-dried solids were crystallised from aqueous methanol and then from chloroform–methanol, to give the *dibromo-ketone*

(6·1 g.), tablets, m. p. 110—113° (decomp.), $[\alpha]_D^{20}$ −89·5° (*c*, 1·1) (Found: C, 51·4; H, 6·9. $C_{20}H_{30}O_2Br_2$ requires C, 51·9; H, 6·5%).

5α: 6β-*Dibromo*-2β-*chloro*-17β-*hydroxy*-17α-*methylandrostan*-3-*one* (V*c*; R = Cl) *and its* 2α-*Chloro-epimer* (VI*c*; R = Cl).—The foregoing compound (6·5 g.) in acetic acid (140 ml.) was treated with chlorine (1 g.) in acetic acid (28 ml.), absorption of halogen being complete in 12 min. The mixture was poured into water and the product isolated with ether. The insoluble fraction (4·9 g., m. p. 120—121°) obtained by trituration of the material with a small volume of cold acetone was purified from aqueous acetone, to give the *dibromo*-2β-*chloro-ketone*, prisms, m. p. 130° (decomp.), $[\alpha]_D^{22}$ −34° (*c*, 1·04) (Found: C, 48·4; H, 5·9. $C_{20}H_{29}O_2ClBr_2$ requires C, 48·4; H, 5·9%). The 2α-*chloro-epimer*, plates (from aqueous ethanol), m. p. 135—136° (decomp.), $[\alpha]_D^{21}$ −72·5° (*c*, 1·2) (Found: C, 48·0; H, 5·6%), was obtained if the mixture was kept overnight.

2α-*Chloro*-17α-*methyltestosterone* (VIII*c*; R = Cl).—Sodium iodide (7 g.) was added to a suspension of the foregoing dibromo-2β-chloro-ketone (5 g.) in acetone (70 ml.). The mixture was stirred for 5 min., then poured into water, and the product extracted with ether. The extract was washed with aqueous sodium thiosulphate and water, dried, and concentrated to 150 ml. After addition of acetic acid (75 ml.) and concentrated hydrochloric acid (3 ml.), the mixture was stored at 0° for 18 hr. The product, isolated with ether, was purified from aqueous methanol, to give 2α-*chloro*-17α-*methyltestosterone* (2·1 g.), plates, m. p. 154—155° (sinters 100—110°), $[\alpha]_D^{23}$ +79° (*c*, 1·08), $\lambda_{max.}$ 243 mμ (4·10) (Found: C, 67·4; H, 8·8. $C_{20}H_{29}O_2Cl,H_2O$ requires C, 67·7; H, 8·8%). The crystals became opaque and then yellow on brief drying at 100°.

17β-*Acetoxy*-5α: 6β-*dibromoandrostan*-3-*one* (IV*b*).—17β-Acetoxyandrost-5-en-3β-ol (3·3 g.) in acetic acid (100 ml.) was treated with bromine (1·6 g.) in acetic acid (10 ml.). Several minutes later, chromium trioxide (1 g.) in 80% acetic acid (10 ml.) was added, and the mixture kept for 18 hr. The solids obtained on precipitation with water were crystallised from aqueous ethanol. The *dibromo-ketone* (2·9 g.) formed plates, m. p. 98—100° (decomp.), $[\alpha]_D^{21}$ −72° (*c*, 1·25) (Found: C, 51·7; H, 5·8. $C_{21}H_{30}O_3Br_2$ requires C, 51·4; H, 6·2%).

17β-*Acetoxy*-5α: 6β-*dibromo*-2β-*chloroandrostan*-3-*one* (V*b*; R = Cl).—The foregoing compound (9·1 g.) in acetic acid (150 ml.) was treated with chlorine (1·44 g., 1·1 mol.) in acetic acid (25 ml.), absorption of halogen being accompanied by the separation of crystals. After 10 min., the mixture was diluted with water and the solids were washed and air-dried. Crystallised from chloroform–methanol, the *dibromochloro-ketone* (7·3 g.) formed needles, m. p. 118° to 130° (decomp.), depending on the rate of heating, $[\alpha]_D^{22}$ −24° (*c*, 1·02) (Found: C, 47·5; H, 5·1. $C_{21}H_{29}O_3ClBr_2$ requires C, 48·1; H, 5·6%).

2α-*Chlorotestosterone Acetate* (VIII*b*; R = Cl).—Prepared from the foregoing compound (5 g.) by the method employed for the preparation of 2α-chloro-17α-methyltestosterone (above), 2α-*chlorotestosterone acetate* (2·7 g.) formed needles (from methanol), m. p. 199—200°, $[\alpha]_D^{22}$ +90° (*c*, 1·22), $\lambda_{max.}$ 242 mμ (4·14) (Found: C, 69·0; H, 7·9. $C_{21}H_{29}O_3Cl$ requires C, 69·1; H, 8·0%).

2α-*Bromo*-17α-*methyltestosterone* (VIII*c*; R = Br).—Bromine (4 g.) in acetic acid (40 ml.) was added to the dibromo-compound (IV*c*) (11·4 g.) in acetic acid (300 ml.), absorption being complete in 5 min., then the mixture was poured into water. The product, isolated with ether, was purified from methylene chloride–hexane, giving prisms (7·5 g.), m. p. 126—128° (decomp.). This material was partially debrominated with sodium iodide (10 g.) in acetone (80 ml.), and the product crystallised from aqueous ethanol, to give prisms (2·7 g.), m. p. 108—110°. Treatment of the latter compound with hydrochloric acid in ether–acetic acid gave a product which was triturated with cold methanol. The insoluble fraction (2·2 g.) on purification from aqueous ethanol gave 2α-*bromo*-17α-*methyltestosterone*, needles, m. p. 155° (decomp.), $[\alpha]_D^{22}$ +90·5° (*c*, 0·86), $\lambda_{max.}$ 244 mμ (4·15) (Found: C, 62·9; H, 7·6. $C_{20}H_{29}O_2Br$ requires C, 63·0; H, 7·7%).

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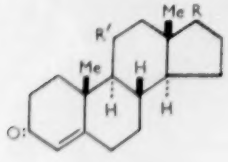
## 247. *Modified Steroid Hormones. Part III.* Some 4-Chloro-3-oxo-Δ⁴-derivatives.*

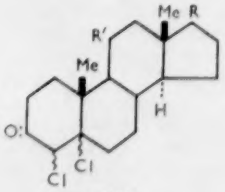
By D. N. Kirk, D. K. Patel, and V. Petrow.

Chlorination of some 3-oxo-Δ⁴-steroids (I) in ether–propionic acid leads to the formation of 4ξ : 5ξ-dichlorides (II), which pass readily under the influence of basic reagents into the corresponding 4-chloro-3-oxo-Δ⁴-steroids (III). The latter may also be obtained directly from the ketones (I) by chlorination in the presence of proton acceptors such as pyridine, dimethylformamide, and ethylene or propylene oxide.

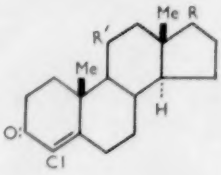
When our studies of the halogenation of steroid hormones began (1954), bromination of 3-oxo-Δ⁴-steroids in ether–acetic acid was known to occur by allylic substitution.[1] On bromination in the presence of proton acceptors, however, we observed formation of 4-bromo-3-oxo-Δ⁴-steroids (see Part I [2]). Concurrent studies on the chlorination of 3-oxo-Δ⁴-steroids (I) are now reported.



(I)



(II)



(III)

(*a*) R = , R′ = $H_2$.

(*b*) R = , R′ = $H_2$.

(*c*) R = :O, R′ = $H_2$.

(*d*) R = , R′ = $H_2$.

(*e*) R = , R′ = $H_2$.

(*f*) R = , R′ = .

(*g*) R = , R′ = $H_2$.

(*h*) R = , R′ = :O.

(*i*) R = , R′ = :O.

(*j*) R = , R′ = $H_2$.

Chlorination of cholest-4-en-3-one (I*a*) in ether–propionic acid led to a dichloride, $C_{27}H_{44}OCl_2$, spectroscopically transparent from 220 to 300 mμ and hence formulated as 4ξ : 5ξ-dichlorocholestan-3-one (II*a*). Reaction of this compound with basic reagents such as pyridine led to formation of a new αβ-unsaturated ketonic product, $C_{27}H_{43}OCl$, which differed from the known 2- and 6-chlorocholest-4-en-3-one [3,4] and is consequently assigned the constitution 4-chlorocholest-4-en-3-one (III*a*).[5] This structure is established by (i) the ultraviolet absorption which reaches a maximum at 256 mμ [α-chlorination of an αβ-unsaturated ketone ($\lambda_{max.}$ 240 mμ) is known [3] to result in a bathochromic shift of the absorption maximum of *ca.* 16 mμ (cf. also Part I [2])], and (ii) its reaction with *o*-phenylenediamine in acetic acid solution to give the quinoxaline derivative [6] of cholestane-3 : 4-dione. Extension of the reaction to the steroid hormones (I*b*—*i*) led to the formation of the corresponding chlorides (II), severally converted into the 4-chloro-hormones (III*b*—*i*).

* Part II, preceding paper.

[1] Djerassi, Rosenkranz, Romo, Kaufmann, and Pataki, *J. Amer. Chem. Soc.*, 1950, **72**, 4534.
[2] Part I, *J.*, 1956, 627.
[3] Ellis and Petrow, *J.*, 1953, 3869.
[4] Barton and Miller, *J. Amer. Chem. Soc.*, 1950, **72**, 370.
[5] Cf. the preparation of 4-bromotestosterone reported by Bremer, Congress Handbook, XIVth Internat. Congr. Pure and Applied Chemistry, p. 162. This abstract became available after completion of our work.
[6] Butenandt, Schramm, Wolff, and Kudszus, *Ber.*, 1936, **69**, 2779; Inhoffen, *ibid.*, 1937, **70**, 1695.

The facility with which the dichloro-compounds (II) pass into the monochloro-compounds (III) led us to examine the chlorination of the unsaturated ketones (I) in the presence of proton acceptors such as pyridine, dimethylformamide, and ethylene and propylene oxide. In parallel with results recorded in Part I[2] on the bromination of 3-oxo-$\Delta^4$-steroids, chlorination gave the 4-chloro-hormones in good yields. As the $4\xi : 5\xi$-dichlorides (II) proved stable to dimethylformamide and ethylene and propylene oxide, the reaction under these experimental conditions evidently proceeds by direct substitution and not by an addition-dehydrochlorination mechanism.

## Experimental

Ultraviolet absorption spectra were kindly determined by Mr. M. T. Davies, B.Sc. (figures in parentheses are log $\varepsilon$). Optical rotations were measured for $CHCl_3$ solutions in a 1 dm. tube.

*Preparation of* $4\xi : 5\xi$-*Dichlorides* (II).—The steroid, dissolved in *ca.* 50 vols. ether and/or dioxan, was treated at 0° to −30° with chlorine in propionic acid (1·05—1·1 mols. of *ca.* M-solution), and the cooled mixture stored in the dark for *ca.* 12—16 hr., then poured into water, and the product isolated with ether and purified by crystallisation.

*Preparation of* 4-*Chloro*-3-*oxo*-$\Delta^4$-*derivatives* (III).—(*a*) The $4\xi : 5\xi$-dichloride (II) was dissolved in pyridine by gentle warming and the solution kept at room temperature for several hr. The product was isolated with ether, the ethereal solution washed with dilute hydrochloric acid and with water, and dried, and the solvent removed. The 4-chloro-steroid was purified by crystallisation.

(*b*) The steroid, dissolved in ethylene oxide (or propylene oxide) (*ca.* 10 vols.) and ether and/or dioxan (10—15 vols.), was treated at 0 to −30° with chlorine in propionic acid (1·05—1·1 mols. of *ca.* M-solution). After being kept in the dark for some hours the mixture was poured into water, and the 4-chloro-steroid isolated with ether and purified by crystallisation.

(*c*) As (*b*), but with a solution of the steroid in dimethylformamide.

(*d*) As (*b*), but with a solution of the steroid in pyridine.

$4\xi : 5\xi$-*Dichlorocholestan*-3-*one* (II*a*) crystallised from ether–methanol (1 : 4) in thick needles, m. p. 120—122°, $[\alpha]_D^{18}$ −4° (*c*, 0·46) (Found : C, 71·3; H, 9·7; Cl, 15·7. $C_{27}H_{44}OCl_2$ requires C, 71·2; H, 9·7; Cl, 15·6%).

4-*Chlorocholest*-4-*en*-3-*one* (III*a*) separated from ether–methanol (1 : 2) in needles, m. p. 126—127, $\lambda_{max.}$ 256 mμ (4·15) (in propan-2-ol), $[\alpha]_D^{20}$ +106° (*c*, 0·492) (Found : C, 77·8; H, 10·2; Cl, 8·6. $C_{27}H_{43}OCl$ requires C, 77·4; H, 10·3; Cl, 8·5%).

The quinoxaline derivative was prepared by heating the foregoing compound (500 mg.) with *o*-phenylenediamine (125 mg.) in acetic acid (5 ml.) under reflux for 2 hr., after which methanol (5 ml.) was added and the solution allowed to crystallise overnight at 0°. The derivative formed pale brown plates, m. p. 205—207°, $\lambda_{max.}$ 239 (4·48) and 321 mμ (4·00) (in EtOH) (Found : N, 6·0. Calc. for $C_{33}H_{43}N_2$ : N, 5·9%), after crystallisation from ethyl acetate. The m. p. was not depressed in admixture with an authentic specimen.[6]

$4\xi : 5\xi$-*Dichloro*-4 : 5-*dihydrotestosterone propionate* (II*b*) formed needles, m. p. 143—144°, $[\alpha]_D^{24}$ −22° (*c*, 0·47) (Found : C, 63·5; H, 8·1; Cl, 16·8. $C_{22}H_{32}O_3Cl_2$ requires C, 63·5; H, 7·8; Cl, 17·1%), after crystallisation from methanol.

4-*Chlorotestosterone propionate* (III*b*) formed needles, m. p. 164°, $\lambda_{max.}$ 254 mμ (4·12) (in propan-2-ol), $[\alpha]_D^{23}$ +114° (*c*, 0·4) (Found : C, 69·8; H, 8·2; Cl, 9·1. $C_{22}H_{31}O_3Cl$ requires C, 69·8; H, 8·3; Cl, 9·4%), after crystallisation from methanol.

$4\xi : 5\xi$-*Dichloroandrostane*-3 : 17-*dione* (II*c*) separated from methylene chloride–methanol in plates, m. p. 187—188°, $[\alpha]_D^{25}$ +40° (*c*, 0·511) (Found : C, 63·4; H, 7·3; Cl, 20·2. $C_{19}H_{26}O_2Cl_2$ requires C, 63·8; H, 7·3; Cl, 19·8%).

4-*Chloroandrost*-4-*ene*-3 : 17-*dione* (III*c*) formed crystals, m. p. 180—182°, $\lambda_{max.}$ 254—255 mμ (4·19) (in propan-2-ol), $[\alpha]_D^{24}$ +206° (*c*, 0·42) (Found : C, 70·6; H, 7·8; Cl, 11·5. $C_{19}H_{25}O_2Cl$ requires C, 71·1; H, 7·9; Cl, 11·1%), after crystallisation from methanol.

$4\xi : 5\xi$-*Dichloro*-4 : 5-*dihydro*-17$\alpha$-*methyltestosterone* (II*d*) crystallised from methanol in needles, m. p. 167°, $[\alpha]_D^{23}$ −26° (*c*, 0·409) (Found : C, 63·3; H, 8·2; Cl, 19·2. $C_{20}H_{30}O_2Cl_2$ requires C, 64·3; H, 8·0; Cl, 19·0%).

4-*Chloro*-17$\alpha$-*methyltestosterone* (III*d*), crystallised from acetone–hexane, had m. p. 142—143°, $[\alpha]_D^{22}$ +99° (*c*, 0·387), $\lambda_{max.}$ 255—256 mμ (4·14) (in propan-2-ol) (Found : C, 71·1; H, 8·9; Cl, 10·7. $C_{20}H_{29}O_2Cl$ requires C, 71·3; H, 8·7; Cl, 10·5%).

$4\xi : 5\xi$-*Dichloro*-4 : 5-*dihydroprogesterone* (II*e*) formed needles, m. p. 172—174°, $[\alpha]_D^{25}$ +46°

(*c*, 0·354) (Found : C, 65·1; H, 8·1; Cl, 18·4. $C_{21}H_{30}O_3Cl_2$ requires C, 65·4; H, 7·8; Cl, 18·4%), after crystallisation from methylene chloride–methanol.

4-*Chloroprogesterone* (III*e*) separated from methanol in rods, m. p. 218—220·5°, $\lambda_{max.}$ 255 mμ (4·125) (in propan-2-ol), $[\alpha]_D^{25}$ +198° (*c*, 0·569) (Found : C, 71·8; H, 8·3; Cl, 10·8. $C_{21}H_{29}O_2Cl$ requires C, 72·3; H, 8·4; Cl, 10·2%).

4ξ : 5ξ-*Dichloro*-11α-*hydroxypregnane*-3 : 20-*dione* (II*f*) crystallised from aqueous acetone in needles, m. p. 174—175°, $[\alpha]_D^{24}$ +25° (*c*, 0·459) (Found : C, 63·0; H, 7·7; Cl, 17·8. $C_{21}H_{30}O_3Cl_2$ requires C, 62·9; H, 7·5; Cl, 17·7%).

4-*Chloro*-11α-*hydroxypregn*-4-*ene*-3 : 20-*dione* (III*f*) formed needles, m. p. 181—183°, $\lambda_{max.}$ 256 mμ (4·12) (in propan-2-ol), $[\alpha]_D^{24}$ +163° (*c*, 0·637) (Found : C, 68·7; H, 7·7; Cl, 10·3. $C_{21}H_{29}O_3Cl$ requires C, 69·0; H, 8·0; Cl, 9·7%).

4ξ : 5ξ-*Dichloro*-21-*acetoxypregnane*-3 : 20-*dione* (II*g*) separated from methylene chloride–methanol (1 : 4) in needles, m. p. 185—186°, $[\alpha]_D^{24}$ +55° (*c*, 0·717) (Found : C, 62·1; H, 7·3; Cl, 16·3. $C_{23}H_{32}O_4Cl_2$ requires C, 62·3; H, 7·3; Cl, 16·0%).

21-*Acetoxy*-4-*chloropregn*-4-*ene*-3 : 20-*dione* (III*g*) crystallised from methylene chloride–methanol (1 : 10) in needles, m. p. 183—184°, $\lambda_{max.}$ 255 mμ (4·12) (in EtOH), $[\alpha]_D^{24}$ +191° (*c*, 0·662) (Found : C, 67·4; H, 7·8; Cl, 8·6. $C_{23}H_{31}O_4Cl$ requires C, 67·9; H, 7·7; Cl, 8·7%).

4ξ : 5ξ-*Dichloropregnane*-3 : 11 : 20-*trione* (II*h*) crystallised from methylene chloride–methanol as needles, m. p. 189—190°, $[\alpha]_D^{25}$ +48° (*c*, 0·605) (Found : C, 62·8; H, 6·9; Cl, 18·4. $C_{21}H_{28}O_3Cl_2$ requires C, 63·2; H, 7·1; Cl, 17·8%).

4-*Chloropregn*-4-*ene*-3 : 11 : 20-*trione* (III*h*) separated from methylene chloride–methanol (1 : 10) in needles, m. p. 198—200°, $\lambda_{max.}$ 257 mμ (4·12) (in EtOH), $[\alpha]_D^{24}$ +275° (*c*, 0·654) (Found : C, 69·8; H, 7·8; Cl, 9·6. $C_{21}H_{27}O_3Cl$ requires C, 69·5; H, 7·5; Cl, 9·8%).

4ξ : 5ξ-*Dichloro*-4 : 5-*dihydrocortisone acetate* (II*i*) had m. p. 235—236° (decomp.), $[\alpha]_D^{24}$ +57° (*c*, 0·211 in dioxan) (Found : C, 58·1; H, 6·6; Cl, 14·9. $C_{23}H_{30}O_6Cl_2$ requires C, 58·3; H, 6·4; Cl, 15·0%), after purification from acetone–ether.

4-*Chlorocortisone acetate* (III*i*) separated from acetone–hexane (1 : 3) in plates, m. p. 232—234° (decomp.), $\lambda_{max.}$ 253 mμ (4·11) (in EtOH), $[\alpha]_D^{24}$ +214° (*c*, 0·202 in dioxan) (Found : C, 63·8; H, 7·1; Cl, 7·8. $C_{23}H_{29}O_6Cl$ requires C, 63·2; H, 6·7; Cl, 8·1%).

4-*Chloro*-17α-*hydroxypregn*-4-*ene*-3 : 20-*dione* (III*j*) separated from methylene chloride–methanol (1 : 10) in needles, m. p. 216—218°, $\lambda_{max.}$ 255 mμ (4·12) (in EtOH), $[\alpha]_D^{26}$ +102° (*c*, 0·98) (Found : C, 68·9; H, 8·3; Cl, 10·2. $C_{21}H_{29}O_3Cl$ requires C, 69·0; H, 8·0; Cl, 9·7%).

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## 248. *The Polarography of Niobium.*

By D. J. Ferrett and G. W. C. Milner.

The polarographic behaviour of niobium in various complexing solutions has been studied with conventional polarographic equipment and with the square-wave polarograph. Previous work in sulphuric, hydrochloric, tartaric, and oxalic acid has been re-investigated. Polarographic steps for niobium have been found in a new series of complexing solutions, *e.g.*, malic, lactic, gluconic, and citric acid. The polarography of niobium in ethylenediaminetetra-acetic acid solutions has been studied in detail. Of these complexes, that of niobium citrate is the most stable to hydrolysis and is easily prepared. The step at −0·86 v (*vs.* S.C.E.) from 2M-citric acid solutions of pH 1 can be used for the determination of from 0·1 to at least 250 μg. of niobium per ml. This step is free from interference by manganese, nickel, chromium, iron, or tungsten, but antimony, titanium, or molybdenum interferes.

Far less is known of the polarography of niobium than of many other transition metals. This is probably due to the difficulty of preparing stable solutions with this element. Niobium salts are readily hydrolysed in solution and this can be prevented only if care is

taken that the solutions are strongly acid or, if the acidity is low, that an excess of a suitable complexing agent is present.

The first polarographic study of niobium was made by Zeltzer,[1] and of his conclusions the only one that has been substantiated is that a fairly well-defined step is obtained in M-nitric acid. The work of Stromberg and Reinus [2] and of Dhar [3] has shown, however, that this step is associated with catalytic phenomena and that its definition depends on the nitrate- and hydrogen-ion concentrations of the solution. The chemical difficulties mentioned above may also be responsible for Stromberg and Reinus's failing to detect steps for niobium in sulphuric, hydrochloric, tartaric, and oxalic acid solutions. Subsequently, Krylov, Kolebatova, and Samarina [4] have found a step in sulphuric acid solutions, Cozzi and Vivarelli [5,6] have reported reductions in hydrochloric acid solutions, and Elson [7] has claimed that steps are produced in tartaric and oxalic acid solutions.

A survey of these base solutions, together with others which appeared to be more suitable for polarographic and analytical studies of niobium,[8] has been made both with conventional polarographic equipment and with the square-wave polarograph.

## Experimental

*Apparatus.*—Conventional polarograms were recorded with an automatic pen-recording polarograph incorporating a Brown Electronik potentiometer constructed in this laboratory.[9] The equipment used for recording square-wave polarograms has already been described.[10]

*Procedure.—Hydrochloric acid.* Cozzi and Vivarelli [5] have shown that $Nb^{5+}$ is reduced to $Nb^{4+}$ in 11M-hydrochloric acid. The waves produced are fairly well defined, but the definition is improved by addition of ethylene glycol. Their investigations with a large mercury cathode [6] have shown that a number of reactions occur as a result of disproportionation of the reduced species.

We have found that suitable solutions for the polarographic investigation of these niobium chloride complexes may be prepared by dissolving freshly precipitated niobium hydroxide in concentrated hydrochloric acid. From these solutions a step with a half-wave potential of −0·54 V against the mercury-pool anode was obtained. The plateau of this step is obscured by the hydrogen reduction wave but a well-defined and separated peak is observable on the square-wave polarogram (see Fig. 1). The shape of this peak indicates, however, that the reduction is not fully reversible. In agreement with the results of Cozzi and Vivarelli, we found that the polarographic step has increased definition when the solution contains 20% ethylene glycol.

It is apparent that at these high hydrogen-ion and chloride-ion concentrations, the competition between the chloride ion and the hydroxide ion is very much in favour of the formation of the niobium chloride complex. The strong affinity of the hydroxyl group for niobium is shown by the fact that only very poorly defined steps are found in 3M-hydrochloric acid solutions even when the chloride-ion concentration is raised to 20M by the addition of calcium chloride solutions. Kanzelmeyer and Freund's spectrophotometric studies [11] have shown a similar relation between the pH and the formation of the niobium chloride complex.

*Sulphuric acid.* Krylov, Kolebatova, and Samarina [4] have shown that solutions of niobium in 70% sulphuric acid yield steps corresponding to the reduction of niobium at the dropping-mercury electrode. The polarograms obtained by us under these conditions, like those reproduced by these authors, reveal poorly defined waves. The half-wave potential of this apparently irreversible reduction is about −1·07 V *vs.* the standard calomel electrode (S.C.E.). No peak can be resolved with the square-wave polarograph.

We have found that the definition of this step, like that of the niobium chloride complex, deteriorates rapidly with decreasing acidity and no step can be detected in 40% sulphuric acid.

[1] Zeltzer, *Coll. Czech. Chem. Comm.*, 1932, **4**, 319.
[2] Stromberg and Reinus, *Z. Phys. Khim. S.S.S.R.*, 1946, **20**, 693.
[3] Dhar, *Analyt. Chim. Acta*, 1954, **11**, 289.
[4] Krylov, Kolebatova, and Samarina, *Doklady Akad. Nauk S.S.S.R.*, 1954, **158**, 593.
[5] Cozzi and Vivarelli, *Ricerca sci.*, 1953, **23**, 2244.
[6] *Idem, Z. Elektrochem.*, 1954, **58**, 177.
[7] Elson, *J. Amer. Chem. Soc.*, 1953, **75**, 4193.
[8] Ferrett and Milner, *Nature*, 1955, **175**, 477.
[9] Whittem and Milner, Atomic Energy Research Establishment, Chemistry Memorandum 185 (1953).
[10] Ferrett and Milner, *Analyst*, 1955, **80**, 132.
[11] Kanzelmeyer and Freund, *Analyt. Chem.*, 1953, **25**, 1807.

Here, once again, reducible species only exist when the hydroxyl-ion concentation is very low. Spectrophotometric studies indicate that these concentrations of niobium (50 μg./ml.) in 40% sulphuric acid are still completely in solution. It appears that only the fully sulphated niobium complex can be reduced at the dropping-mercury electrode, and that when any of the sulphate groups is replaced by a hydroxyl group the complex is too stable to be reduced in the voltage range available for polarographic studies. It is clear that this step could find only a limited use in polarographic analysis, and this system was, therefore, not studied further.

*Tartaric acid.* Niobium forms complexes with tartaric acid and these can most readily be formed by extracting the melt formed by the bisulphate fusion of niobium pentoxide with an aqueous solution of tartaric acid. It has been shown [12] that solutions produced in this manner

FIG. 1. *Square-wave polarogram for niobium in* 10M-HCl (Nb = 10 μg./ml.).

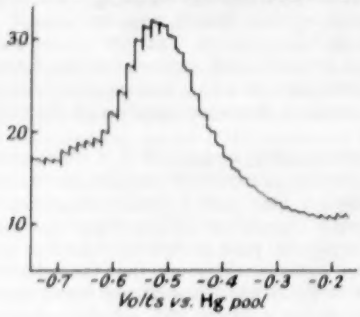
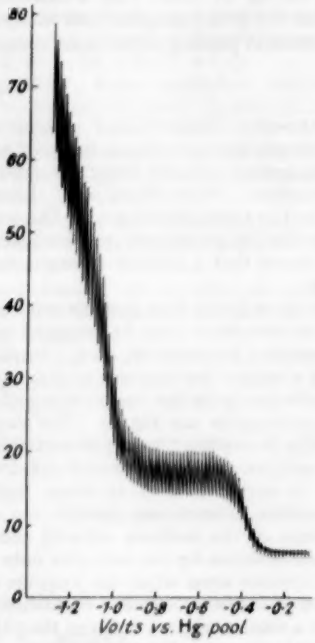



FIG. 2. *Polarogram for niobium* in 0·1M-EDTA, pH 3·1 (Nb = 150 μg./ml.).




yield a niobium step with a half-wave potential at about −1·0 v *vs.* the mercury-pool anode. We have confirmed the existence of this step. It is best defined in 0·5M-solutions of tartaric acid at pH 1·5 (see Fig. 3). Even so, the step proved unsuitable for further study.

Elson [7] has reported that niobium–tartrate complexes formed by the action of tartaric acid on precipitated niobium hydroxide produce steps with half-wave potentials in the range −1·96 to −2·00 v *vs.* S.C.E. According to our experience this method of preparing solutions proved unreliable for quantitative work, some of the niobium often remaining undissolved. We also failed to confirm the existence of steps for niobium in the potential region cited by this worker. It is noteworthy that Elson reported polarographic steps for tantalum from similar base solutions. Again, we were unable to find reduction steps for this element or to hold the tantalum satisfactorily in solution under the reported experimental conditions.

*Oxalic acid.* Elson has reported that niobium in oxalate solutions gives steps with half-wave potentials of −1·53 and −2·18 v *vs.* S.C.E., corresponding to the reduction to the $Nb^{4+}$ and the $Nb^{3+}$ state, respectively. The most convenient procedure for preparing oxalate solutions of niobium consists in fusing niobium pentoxide with potassium hydrogen sulphate and extracting

[12] Mrs. B. Lamb, personal communication.

FIG. 3. *Square-wave polarogram for niobium in* 0·1M-EDTA, pH 3·4 (Nb = 15 μg./ml.).

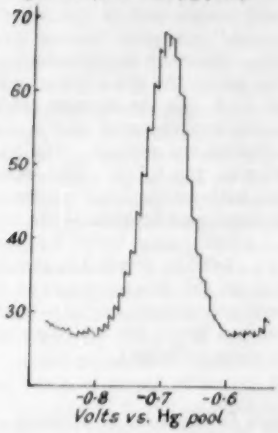



FIG. 4. *Square-wave polarogram for second niobium wave in* 0·2M-EDTA, pH 5·0 (Nb = 15 μg./ml.).

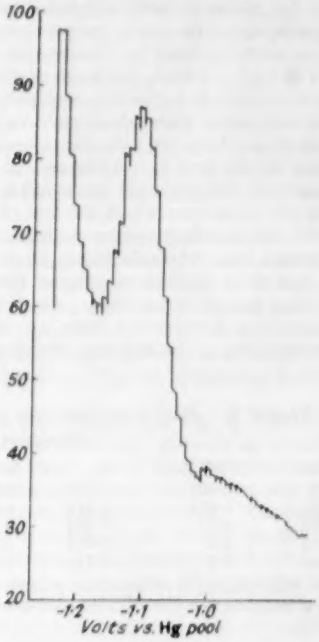



FIG. 5. *Polarogram for niobium in* 2M-*citric acid*, pH 3·7 (Nb = 250 μg./ml.).

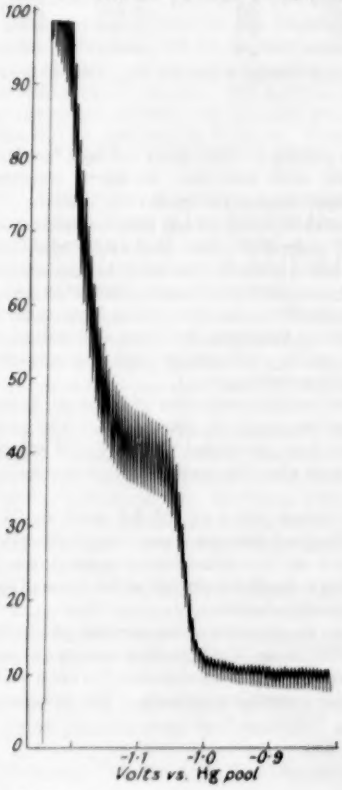
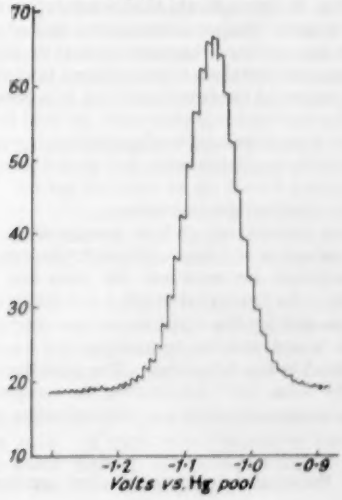



FIG. 6. *Square-wave polarogram for niobium in* 2M-*citric acid*, pH 3·7 (Nb = 25 μg./ml.).



the melt with an aqueous solution of oxalic acid. With solutions so prepared, we failed to observe any steps for the reduction of niobium at the dropping mercury electrode.

*Ethylenediaminetetra-acetic acid* (EDTA). Experiments showed that a stable complex is formed by niobium with ethylenediaminetetra-acetic acid under certain pH conditions.[9] Satisfactory solutions can be prepared as follows: "Spec-pure" potassium niobate (40 mg.) is dissolved with warming in M-potassium hydroxide (15 ml.). Disodium ethylenediaminetetra-acetate (3·7 g.) is added, the whole is diluted to 90 ml. with water, the pH adjusted with 10% (v/v) sulphuric acid to the required value within the range 3—6, and the mixture made up to 100 ml. with water (this solution is 0·1M in EDTA). Solutions thus prepared give well-defined niobium steps:[9] at pH 3·05, for example (see Fig. 2), two steps are evident. The half-wave potential of the first is −0·609 and of the second −1·05 v *vs.* the S.C.E. The second step coalesces with the hydrogen wave and is difficult to measure with conventional equipment.

The pH range over which the first of these two steps is developed depends at the lower end upon the ethylenediaminetetra-acetic acid concentrations. At pH values below 3·0, the acid is precipitated from 0·1M-solutions. More dilute solutions, *e.g.*, 0·0001M at pH 1·0, give niobium steps, but these conditions become less suitable for study as the concentration of the acid approaches that of the niobium. At pH 6 and higher, the niobium complex begins to break down and niobium is precipitated from solution. Between these two limits the half-wave potential and reversibility of the first step at different pH values are given in Table 1.

TABLE 1. *Half-wave potential and reversibility data for niobium in solutions of ethylenediaminetetra-acetic acid.*

| pH | [EDTA] | $E_{\frac{1}{2}}$ | Slope * | pH | [EDTA] | $E_{\frac{1}{2}}$ | Slope * |
|---|---|---|---|---|---|---|---|
| 1·1 | 0·001 | −0·427 | 0·065 | 3·40 | 0·1 | −0·615 | 0·069 |
| 2·0 | 0·001 | −0·419 | 0·060 | 3·40 | 0·02 | −0·623 | 0·066 |
| 2·95 | 0·01 | −0·572 | 0·064 | 3·60 | 0·1 | −0·657 | 0·072 |
| 3·05 | 0·1 | −0·609 | 0·059 | 4·05 | 0·1 | −0·767 | 0·100 |
| 3·20 | 0·1 | −0·613 | 0·062 | 4·75 | 0·1 | −0·835 | 0·086 |

* This column represents the inverse slope of the applied voltage v against $\log_{10} i/(i_d - i)$ graph and is equal to $0{\cdot}059/n$ for a reversible $n$-electron change.

The variation of the half-wave potential with pH is similar to that observed by Pecsok and Maverick [13] for the titanium–ethylenediaminetetra-acetic acid complex. It seems reasonable to suppose that the mechanism for the formation of these two complexes is very similar.

The slope for a reversible one-electron reduction should be 0·059 and it would appear, therefore, that the first step is due to the reduction $Nb^{5+} \longrightarrow Nb^{4+}$ and that this reduction is reversible at pH 3·2 and below. The polarograms obtained with the square-wave polarograph (see Fig. 3) also indicate that the reaction is a reversible one-electron change. For example, the peak-height obtained is similar to that obtained from a thallium solution of the same molarity, while the width of the peak at half its maximum height is the same for these two reductions. Barker and Faircloth [14] have shown that the half-width of the peak can be used as a measure of the number of electrons involved in a completely reversible reduction.

The conventional step could be used for the quantitative determination of niobium, since its height is proportional to the niobium concentration over the range 5—500 μg./ml. The greater sensitivity available with the square-wave polarograph has permitted the study of solutions containing 0·1—5 μg. of niobium per ml. Over this range also, the peak height is proportional to the niobium concentration.

The second step is best developed in solutions of higher pH, *e.g.*, pH 5·0 with an EDTA concentration of 0·2M. Although the step coalesces with the hydrogen wave when conventional polarograms are recorded, the peak can be clearly seen on the square-wave polarogram (see Fig. 4). In the region of pH 4 and 5 this second step has a small maximum which cannot easily be removed by the usual maximum suppressors (*e.g.*, gelatin, fuchsin).

It is not possible to analyse this second step, so as to determine the number of electrons involved in the reduction. The most likely process is $Nb^{4+} \longrightarrow Nb^{3+}$, so that an overall change of $Nb^{5+} \longrightarrow Nb^{3+}$ occurs over the complete potential range. This mechanism has been verified by a coulometric study of the reduction at a large stirred mercury cathode. The potential of

[13] Pecsok and Maverick, *J. Amer. Chem. Soc.*, 1954, **76**, 358.
[14] Barker and Faircloth, personal communication.

this cathode was automatically controlled at −1·5 v *vs.* S.C.E., and the current that flowed measured by an oxygen–hydrogen coulometer.[15] A solution 0·1M in ethylenediaminetetra-acetic acid at pH 4·0 was electrolysed with nitrogen passing through it until the background current fell to less than 1 mA. Then a small quantity of a solution of niobium in 0·1M-EDTA, containing 38·5 mg. of niobium was carefully added and the electrolysis was continued until the final current decreased to a value less than 1 mA. The total current measured in the coulometer was equivalent to 35 mg. of niobium, an overall two-electron reduction being assumed. After the reduction the solution was brownish-orange, a characteristic of tervalent niobium.

Although the reduction steps produced from these solutions are well defined, they are recommended for analytical use only when it is impossible to use a citrate base solution (see below). The main advantage of the citrate complex arises from its greater stability over the niobium–EDTA complex. Unfortunately, however, suitable citrate solutions can only be prepared from metallic niobium samples.

*Citric acid.* The niobium–citrate complex is most stable if prepared as follows: "Spec-pure" niobium metal (25 mg.), in a platinum crucible, is treated with water (1 ml.), concentrated nitric acid (5 ml.), and concentrated hydrofluoric acid (3 drops) and warmed, if necessary, to effect dissolution. Sulphuric acid (20% v/v, 5 ml.) is added and the solution heated until fumes of this acid appear. Water is added and the solution again heated until fumes appear, to ensure complete removal of fluoride. The solution is diluted slightly with water, treated with citric acid (42 g. in 50 ml. of water), adjusted to pH 1·0 with a 10% w/v potassium hydroxide solution, and diluted to 100 ml. with water.

The solutions prepared from the metal gave excellent polarograms (see Fig. 5). The height of the step was proportional to concentration over the range studied, *viz.*, 250—10 μg. of niobium per ml. Measurement of the slope of the polarographic step showed that the reduction was reversible and that, like the niobium–ethylenediaminetetra-acetic acid complex, it was due to the reduction of $Nb^{5+}$ to $Nb^{4+}$. The square-wave polarogram is shown in Fig. 6. The height of the peak is proportional to the niobium concentration over the range 10—0·1 μg./ml. The solutions were stable for 24 hr. and longer, and the dissolution procedure was always reliable.

The niobium citrate reduction step can be obtained over a wide range of hydrogen-ion and citric acid concentrations. We have studied it from pH 6·0 to solutions 2M in sulphuric acid. Over the range pH 4·0—0·0, the half-wave potential shifts in a manner similar to that observed for the EDTA complex in Table 1. Thus at pH 4·0 the half-wave potential is −1·13 v, and at pH 0 it is −0·75 v *vs.* S.C.E. At pH 1·0, where most of our studies were made, it is −0·86 v *vs.* S.C.E.

The reduction steps obtained from niobium citrate solutions when the citric acid concentration was in the range 0·1—0·4M were not well defined. They tended to coalesce with the hydrogen waves or to have sloping plateaux. Over the citric acid concentration 0·4—2M, the polarograms were well defined; the diffusion current decreases, however, with increasing viscosity of the solution. The conditions finally chosen were 2M-citric acid adjusted to pH 1.

The reduction step obtained in all the solutions studied behaved similarly to the first reduction step found in niobium–ethylenediaminetetra-acetic acid solutions. Measurements of the slope of the rising portion of the step showed that the reduction in citric acid base solutions also involved only one electron. No evidence for a second step was found in citric acid solutions, however.

This step is of analytical value and is well separated from the uranium wave in 2M-citric acid of pH 1·0. Manganese, nickel, tungsten, chromium, and iron also do not interfere, but antimony, titanium, and large quantities of molybdenum do.

*Other α-hydroxy-acids.* Niobium forms complexes with a number of other α-hydroxy-acids, *e.g.*, malic, lactic, gluconic, and tartronic acids. Solutions of the first three gave typical niobium polarograms and were prepared as described for citric acid. Further details of the results obtained are as follows:

Malic acid. The niobium malate complex is reduced to give a single step corresponding to a $Nb^{5+} \longrightarrow Nb^{4+}$ reduction. The malic acid available, however, contained an impurity which gave a reduction wave superimposed upon that of the niobium complex. It proved very difficult to purify this malic acid without laborious treatment, and further study was not carried out.

Lactic acid. Solutions of niobium lactate behaved similarly to those of malic acid, a large step being present, even in "AnalaR" samples of the acid. A triple precipitation of barium

[15] Lingane, "Electro-Analytical Chemistry," Interscience Inc., New York, 1953, p. 349.

lactate, however, reduced this impurity considerably. The reduction in lactic acid solutions corresponds to $Nb^{5+} \longrightarrow Nb^{4+}$ and is proportional in height to the niobium concentration.

Gluconic acid. Niobium gluconate gives polarographic waves that are best defined in the pH range 4—6 in solutions 0·3M in gluconic acid. No interference from impurities in the gluconic acid is noticed here. The slope of the step in this case also corresponds to a $Nb^{5+} \longrightarrow Nb^{4+}$ reduction process.

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## 249. *The Chemistry of* D-*Ribose and its Derivatives. Part VI.** iso*Propylidene and Benzylidene Derivatives.*

By G. R. BARKER and J. W. SPOORS.

The properties of *O-iso*propylideneribose anhydride are consistent with the structure proposed by Levene and Stiller. Condensation of ribose with benzaldehyde yields 2 : 3-*O*-benzylidene-D-ribose and what is believed to be 1 : 5-2 : 3-di-*O*-benzylidene-D-ribose. No anhydride is formed. Similarly 2 : 3-*O-iso*propylidene-D-ribose and benzaldehyde yield 1 : 5-*O*-benzylidene-2 : 3-*O-iso*propylidene-D-ribose. The difference in the course of the reactions with acetone and benzaldehyde is attributed to steric factors.

CONDENSATION of D-ribose with acetone leads to 2 : 3-*O-iso*propylidene-D-ribose and an *iso*propylideneribose anhydride which was formulated by Levene and Stiller as 1 : 5-anhydro-2 : 3-*O-iso*propylidene-D-ribofuranose.[1] No rigorous proof of the constitution of this anhydride was offered but the molecular weight, determined in boiling benzene, was in agreement with the proposed structure. Since we had previously found that the diacetyl anhydride of ribose was dimeric,[2] as opposed to the structure originally proposed,[3] it seemed possible that the *iso*propylideneribose anhydride might be dimeric and that it decomposed in boiling benzene. We have prepared the latter anhydride by an improved method and have redetermined its molecular weight by depression of the freezing point of benzene and by isothermal distillation.[4] These measurements confirmed the monomeric nature of the material and we have therefore sought chemical evidence to support the bicyclic system proposed by Levene and Stiller.

Since hydrolytic conditions which are sufficient to remove the *iso*propylidene residue also destroy the anhydride structure, it was decided to attempt to prepare the analogous 2 : 3-*O*-benzylideneribose anhydride and to remove the benzylidene residue by hydrogenolysis. However, condensation of ribose with benzaldehyde yielded no anhydride but a mono- and a di-*O*-benzylidene-D-ribose. The former we designate as 2 : 3-*O*-benzylidene-D-ribose, since it reduces Fehling's solution and on methylation and hydrolysis yields 5-*O*-methyl-D-ribose. It is not oxidised by sodium metaperiodate in 50% aqueous dioxan, and, since it gives rise to 5-*O*-methyl-D-ribose, appears to exist in the furanose form. The same may be said of 2 : 3-*O-iso*propylidene-D-ribose which we find is also resistant to sodium metaperiodate and yields 5-*O*-methyl-D-ribose. It is of interest that 2 : 3-di-*O*-methyl-D-ribose is exceptional in being oxidised stoicheiometrically,[5] presumably reacting in the acyclic form.

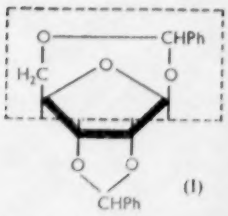
The dibenzylideneribose possesses no free hydroxyl group, as shown by its infrared spectrum in the region 3700—3400 cm.$^{-1}$, and on hydrolysis with dilute mineral acid yields 2 mols. of benzaldehyde. After boiling with Raney nickel in ethanol for 45 min., hydrogenolysis of the benzylidene residues is complete since on paper chromatograms only one spot, corresponding to ribose, is observed. The dibenzylideneribose was treated with

* Part V, *J.*, 1955, 1327.

[1] Levene and Stiller, *J. Biol. Chem.*, 1933, **102**, 187.
[2] G. R. Barker and Lock, *J.*, 1950, 23; G. R. Barker, Jeanloz, and Lock, *Nature*, 1951, **167**, 42.
[3] Bredereck, Köthnig, and Berger, *Ber.*, 1940, **73**, 956.
[4] Niederl, Kasanof, Kisch, and Rao, *Mikrochem. Microchim. Acta*, 1949, **34**, 132.
[5] G. R. Barker and Smith, *J.*, 1955, 1323.

Raney nickel for 15 min. and the total product was methylated with methyl iodide and silver oxide. Hydrolysis and paper chromatography of the methylated material yielded, besides ribose, tri-*O*-methyl-D-ribose, a trace of di-*O*-methyl-D-ribose, and a mono-*O*-methyl-D-ribose which consisted only of 5-*O*-methyl-D-ribose.[6] It thus seems probable that on partial hydrogenolysis the dibenzylideneribose is converted into 2 : 3-*O*-benzylidene-D-ribose, and the dibenzylideneribose is therefore believed to be either 1 : 5-2 : 3-di-*O*-benzylidene-D-ribose or 1 : 4-2 : 3-di-*O*-benzylidene-D-ribose. No final choice can be made between these two possibilities at the moment. Infrared spectra have been used recently to distinguish between furanose and pyranose rings,[7] but the method is not applicable in this case since the bands of most diagnostic value lie too close to those due to the monosubstituted benzene ring. The furanose structure appears to be more likely since the alternative structure involves the adoption of the boat-form by the ribopyranose ring. However, it must be borne in mind that a boat-form six-membered ring is present also in the 2 : 3-*O*-*iso*propylideneribose anhydride. The latter can conceivably be formed by elimination of water either between the 1- and the 4-position of 2 : 3-*O*-*iso*propylidene-D-ribopyranose or between the 1- and the 5-position of 2 : 3-*O*-*iso*propylidene-D-ribofuranose. The latter mode of formation seems to be more likely since 2 : 3-*O*-*iso*propylidene-D-ribose appears to exist in the furanose form (see above). 2 : 3-*O*-Benzylidene-D-ribose under similar conditions also yields only 5-*O*-methyl-D-ribose and appears also to exist in the furanose form. In that case, it would seem more likely that the formation of di-*O*-benzylidene-D-ribose involves condensation with hydroxyl groups at positions 1 and 5.

Whichever of the two structures is adopted for the di-*O*-benzylidene-D-ribose, it remains to be considered why ribose behaves differently in its condensations with acetone and benzaldehyde. We believe this to be due to the inability of the *iso*propylidene residue to bridge the 1 : 5- or 1 : 4-positions, rather than to any effect produced by the group at the 2 : 3-positions. 2 : 3-*O*-*iso*Propylidene-D-ribose condenses readily with benzaldehyde, to give a product which we formulate as 1 : 5-*O*-benzylidene-2 : 3-*O*-*iso*propylidene-D-ribose. Since the condensation is carried out in presence of an excess of benzaldehyde any change in the location of the *iso*propylidene residue must be *via* an intramolecular rearrangement. We do not believe that this occurs, however, since on hydrogenolysis the compound is reconverted into 2 : 3-*O*-*iso*propylidene-D-ribose. Models of 2 : 3-1 : 4- and 2 : 3-1 : 5-di-*O*-benzylidene-D-ribose reveal that the hydrogen atom attached to the acetal carbon atom of the 1 : 4- or 1 : 5-*O*-benzylidene residue is very crowded and cannot be replaced by a methyl group. It thus appears that the formation of a 1 : 4- or 1 : 5-*O*-*iso*propylidene derivative is sterically prevented. This is found to be the case whichever conformation is adopted for the seven-membered ring indicated in (I). Further, models show that a change from one of the stable conformations of this seven-membered ring to the other necessitates changing also the 1 : 4- or 1 : 5-*O*-benzylidene residue from one geometrical form to the other. We have, in fact, obtained two forms of the *O*-benzylidene-*O*-*iso*propylidene-D-ribose which, although direct proof is not at the moment possible, we believe to be geometrical isomers.



(I)

## Experimental

1 : 5-*Anhydro*-2 : 3-O-iso*propylidene*-D-*ribofuranose*.—D-Ribose (3·2 g.) was shaken at approx. 18° for 18 hr. with acetone (150 c.c.), powdered anhydrous zinc chloride (3 g.), and 80% phosphoric acid (1 c.c.). The mixture was neutralised with 20% aqueous sodium hydroxide and extracted repeatedly with chloroform. The extract was washed with water (2 × 50 c.c.) and dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residual gum (4·5 g.) was dissolved in benzene and percolated through a column (18 × 3·5 cm.) of neutral alumina (Grade H; Peter Spence). Elution with 1 : 1 benzene–ether yielded, after removal of the solvent, 1 : 5-anhydro-2 : 3-*O*-*iso*propylidene-D-ribofuranose (0·9 g.) [Found: C, 56·0;

[6] G. R. Barker, Noone, Smith, and Spoors, *J.*, 1955, 1327.
[7] S. A. Barker and Stephens, *J.*, 1954, 4550.

H, 7·4; *M* (cryoscopic in benzene), 169; *M* (isothermal distillation), 183. Calc. for $C_8H_{12}O_4$: C, 55·8; H, 7·0%; *M*, 172]. Subsequent elution with ether containing 20% of methanol yielded, after removal of solvent, 2 : 3-*O*-*iso*propylidene-D-ribose (3·1 g.).

*Condensation of Ribose with Benzaldehyde.*—D-Ribose (3 g.) was shaken at approx. 18° for 20 hr. with freshly distilled benzaldehyde (37 g.) and powdered anhydrous zinc chloride (5 g.). Chloroform (50 c.c.) was added to the mixture which was washed with saturated aqueous sodium hydrogen carbonate, and the chloroform layer was washed with water (2 × 25 c.c.), dried ($MgSO_4$), and concentrated to dryness under reduced pressure. The residue was dissolved in benzene (200 c.c.) and percolated through a column (15 × 3·5 cm.) of alumina (Grade O; Peter Spence). Elution with ether and removal of the solvent yielded 1 : 5-2 : 3-*di*-O-*benzylidene*-D-*ribose* (1·4 g.) which separated from methanol in needles, m. p. 117—119°, $[\alpha]_D^{19}$ +7·3° (*c* 1·6 in $CHCl_3$) (Found: C, 70·05; H, 5·3. $C_{19}H_{18}O_5$ requires C, 69·92; H, 5·5%). Elution of the column with methanol yielded, after removal of solvent, 2 : 3-O-*benzylidene*-D-*ribose* (0·06 g.) which separated from pentane–chloroform (3 : 1) in needles, m. p. 98—101°, $[\alpha]_D^{19}$ −16·5° (*c* 0·605 in MeOH) (Found: C, 60·45; H, 5·6. $C_{12}H_{14}O_5$ requires C, 60·5; H, 5·9%). A solution containing 1 : 5-2 : 3-di-*O*-benzylidene-D-ribose (0·0037 g.) and N-hydrochloric acid (2 c.c.) was diluted to 50 c.c. with ethanol and kept at approx. 18°. At intervals, the optical density of the solution at 245·5 mμ was measured. After 30 hr., the optical density had a constant value of 0·26 which corresponds to the formation of 2·04 moles of benzaldehyde per mole of dibenzylideneribose.

*Methylation and Hydrolysis of* 2 : 3-O-*Benzylidene*-D-*ribose.*—The material (0·065 g.) was refluxed and stirred for 6 hr. with methyl iodide (30 c.c.) and silver oxide (0·25 g.), further quantities of silver oxide (0·25 g.) being added every 0·5 hr. Refluxing and stirring were continued for a further 2 hr., silver salts were removed and washed with ether, and the combined filtrates were concentrated to dryness under reduced pressure. The residual gum was percolated in 1 : 1 benzene–ether through alumina (Grade H, Peter Spence) (10 g.). The eluate was concentrated under reduced pressure to a syrup which was kept at 60° for 2 hr. with 0·1N-hydrochloric acid (25 c.c.). Chloride ions were removed with silver carbonate, the silver salts being washed with methanol. The combined filtrates were concentrated under reduced pressure to a syrup (0·015 g.). A portion (approx. 3 mg.) of this was chromatographed on a large sheet of paper (Whatman No. 1) in butan-1-ol–water. Test strips were sprayed with the aniline phthalate spray reagent and the zone of $R_F$ 0·41 was eluted. The eluate was chromatographed in butan-1-ol–saturated aqueous boric acid and had an $R_F$ value (0·22) identical with that of authentic 5-*O*-methyl-D-ribose.[8] A further portion (0·0086 g.) of the syrup was oxidised with sodium metaperiodate (0·0504 g.) in water (10 c.c.). The material consumed 3 moles of sodium metaperiodate per mole of sugar.

*Hydrogenolysis of* 1 : 5-2 : 3-*Di*-O-*benzylidene*-D-*ribose.*—The material (0·13 g.) was refluxed for 15 min. in ethanol (30 c.c.) with Raney nickel (1·5 g.). After removal of the catalyst, the solution was concentrated to dryness under reduced pressure. The residual gum was refluxed and stirred with methyl iodide (20 c.c.) and silver oxide (0·25 g.) for 6 hr., further quantities of silver oxide (0·25 g.) being added at intervals of 0·5 hr. Silver salts were removed and washed with ether and the combined filtrates were concentrated to dryness under reduced pressure. The residual gum was boiled with 0·04N-hydrochloric acid (10 c.c.) for 1 hr., freed from chloride ions with silver carbonate, and the solution, after being concentrated to a small volume under reduced pressure, was chromatographed on a large sheet of paper (Whatman No. 1) in butan-1-ol–water. A test strip was sprayed with the aniline phthalate spray reagent and the zone containing a fraction at $R_F$ 0·41 was eluted. This material had an $R_F$ value (0·22) identical with authentic 5-*O*-methyl-D-ribose when chromatographed in butan-1-ol–saturated aqueous boric acid.[8]

1 : 5-O-*Benzylidene*-2 : 3-O-iso*propylidene*-D-*ribose.*—2 : 3-*O*-*iso*Propylidene-D-ribose (1·0 g.) was shaken at approx. 19° for 48 hr. with freshly distilled benzaldehyde (25 c.c.) and powdered anhydrous zinc chloride (1·5 g.). The product was isolated by the method used in the condensation of ribose with benzaldehyde and was chromatographed on a column (10 × 3·5 cm.) of alumina (Grade O, Peter Spence). Elution with benzene containing 15% of ether yielded, after removal of solvent, a *benzylidene*iso*propylideneribose* (0·065 g.) which separated from light petroleum in needles, m. p. 134—135°, $[\alpha]_D^{16}$ −59·6° (*c* 0·5 in $CHCl_3$) (Found: C, 64·6; H, 5·6. $C_{15}H_{18}O_5$ requires C, 64·7; H, 6·5%). Elution with benzene containing 50% of ether yielded, after removal of solvent, an (?) isomeric *form* (0·78 g.) which separated from ethanol in needles, m. p.

[8] G. R. Barker and Smith, *Chem. and Ind.*, 1954, 19.

198—200°, $[\alpha]_D^{15}$ −40° (*c* 0·725 in $CHCl_3$) (Found : C, 64·8; H, 5·6. $C_{15}H_{18}O_4$ requires C, 64·7; H, 6·5%).

Treatment of both the above materials with Raney nickel as described above for the hydrogenolysis of di-*O*-benzylidene-D-ribose yielded a product which had an $R_F$ value (0·8) in butan-1-ol–water identical with that of 2 : 3-*O*-*iso*propylidene-D-ribose.

THE UNIVERSITY, MANCHESTER, 13. [*Received, October 17th*, 1955.]

## 250. *The Synthesis and Stereochemistry of Some Tervalent Arsenic Compounds.*

By I. G. M. CAMPBELL and R. C. POLLER.

The synthesis of substituted 9-arsafluorenes by several different routes, and the optical resolution of two of them, are described. The enantiomers of 9-*p*-carboxyphenyl-2-methoxy-9-arsafluorene, $[\alpha]_D$ ±160°, are optically stable at room temperature in pyridine. Racemisation occurs in chloroform–ethanol, slowly at 70° and more rapidly at 111°; at 111° the half-life is 17 min. The catalytic effect of hydrogen chloride on the racemisation is demonstrated and shown to be less pronounced in this series than in the corresponding 9-stibiafluorenes. (+)-2-Amino-9-phenyl-9-arsafluorene possesses high optical stability, for the specific rotation, $[\alpha]_D$ +255° (in ethanol), is unchanged after 1 hour's heating at 110°.

ENANTIOMERS of simple 3-covalent arsenic compounds have not as yet been isolated although, from physical evidence, their configuration is undoubtedly pyramidal, so that compounds in which arsenic is attached to three different groups should be capable of optical resolution. Various values for the H–As–H angle in arsine have been published; one of the most recent, 91·5°, has been calculated by Nielsen from moments of inertia [1] and is in good agreement with that of 92° obtained from microwave spectra.[2] Trimethylarsine and arsenic tribromide have been examined by electron diffraction and the bond angles found to be 96° and 100° respectively.[3] Perhaps of more importance than the bond angle to the practical stereochemist is some estimate of the ease of inversion of these pyramidal molecules, and this has been given by Weston [4] who, from molecular dimensions and vibration frequencies, has calculated the potential-energy barrier to inversion. The results of this calculation are expressed as a temperature at which the half-life of "racemisation" would be 2 hr., and the value found for trimethylarsine is +107° C. If this estimate is valid, racemisation by inversion should be too slow to prevent the resolution of a dissymmetric tertiary arsine.

Nevertheless, all attempts to isolate enantiomers of suitably substituted arsines have failed [5] except in the case of the phenoxarsines,[6] where the dissymmetry may possibly arise from the folding of the molecule. In this investigation we chose to examine substituted 9-arsafluorenes, in which the arsenic atom is held in a chemically stable five-membered ring, because several members of the analogous 9-stibiafluorene series have been obtained in optically active forms,[7] and it seemed likely that the optical and chemical stability of the corresponding arsenic compounds would be even more favourable for resolution experiments.

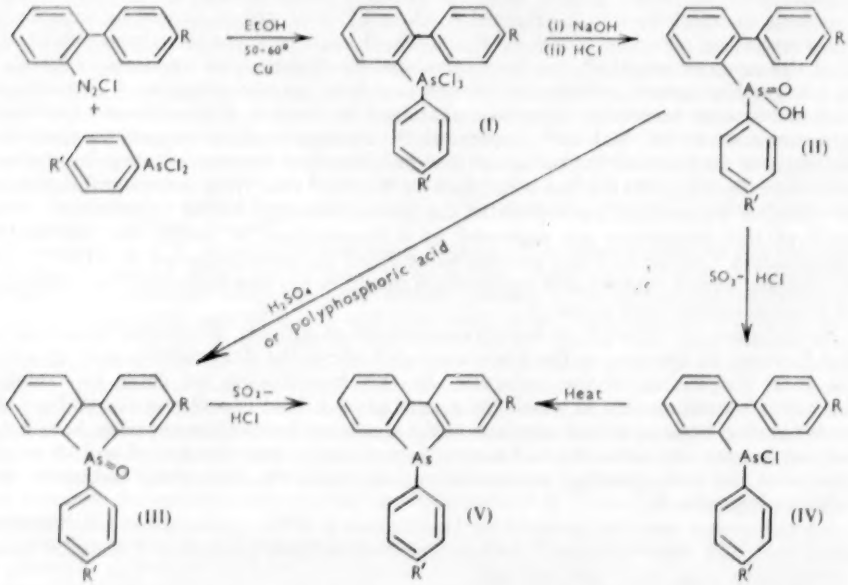
9-Arsafluorenes were first prepared by Aeschlimann *et al.*[8] by cyclisation of 2-diphenylylarsonic acid, and, more recently, Feitelsohn and Petrow,[9] and Garascia and Mattei,[10] have

[1] Nielsen, *J. Chem. Phys.*, 1952, **20**, 1955.
[2] Loomis and Strandberg, *Phys. Rev.*, 1951, **81**, 798.
[3] Allen and Sutton, *Acta Cryst.*, 1950, **3**, 46.
[4] Weston, *J. Amer. Chem. Soc.*, 1954, **76**, 2645.
[5] Kamai, *Ber.*, 1935, **68**, 960, 1893; *J. Gen. Chem.* (*U.S.S.R.*), 1940, **10**, 683; 1942, **12**, 104; 1947, **17**, 2178.
[6] Lesslie and Turner, *J.*, (*a*) 1934, 1170; (*b*) 1935, 1268; 1936, 730; Lesslie, *J.*, 1938, 1001; 1949, 1183.
[7] Campbell and Morrill, *J.*, 1955, 1662.
[8] Aeschlimann, Lees, McLeland, and Nicklin, *J.*, 1925, 66.
[9] Feitelsohn and Petrow, *J.*, 1951, 2279.
[10] Garascia and Mattei, *J. Amer. Chem. Soc.*, 1953, **75**, 4589.

shown that the arsenic ring system can withstand nitration without disruption. In this work, however, we have largely avoided substitution and have used as starting materials suitably substituted 2-aminodiphenyls and proceeded to the 9-arsafluorenes by several different routes. In the first, using standard procedures, 2-aminodiphenyl was converted into 9-chloro-9-arsafluorene [8] and the chlorine atom replaced by the *p*-tolyl group by means of the Grignard reaction. Oxidation by alkaline permanganate left the ring system intact and gave 9-*p*-carboxyphenyl-9-arsafluorene oxide, from which the oxygen was readily removed by reduction with sulphur dioxide.

The second route involved a series of reactions, outlined below, similar to those which had proved successful in the antimony series.[11]

In the preparation of the 9-stibiafluorenes, the diazonium antimony chloride double salt, prepared in aqueous acid, was isolated and caused to interact with an arylstibonous chloride in ethanol, giving the diarylstibinic trichloride corresponding to (I). Attempts to isolate the diazonium arsenic chloride double salt under the same conditions failed but, by addition of the requisite 2-diphenyldiazonium salt, prepared in ethanol under " anhydrous " conditions, to the arylarsonous chloride, a transient yellow salt was formed but rapidly decomposed to give, presumably, the trichloride (I). This trichloride could not be readily isolated in crystalline form, in contrast to the corresponding antimony compounds, but, by hydrolysis of the reaction mixture, the arsinic acid (II) was isolated in yields much higher and more consistent than those obtained under the usual alkaline Bart reaction conditions. The latter process, in our hands, gave unpredictable results despite careful control of pH of the arylarsenite solutions.




Ring closure of the diarylarsinic acids to the 9-arsafluorene oxides (III) was effected by hot concentrated sulphuric acid, except in the case of the 4′-methoxy-derivative (II; R = OMe, R′ = $CO_2H$) where sulphonation occurred. Acetic anhydride containing a little sulphuric acid also failed to cyclise this arsinic acid and thermal elimination of hydrogen chloride from the arsinous chloride (IV; R = OMe, R′ = $CO_2H$), though successful, also removed arsenic to some extent. Polyphosphoric acid, however, proved to

[11] Campbell, *J.*, 1950, 3109; 1952, 4448.

be a most effective cyclising agent in this series, and when the acid (II; R = OMe, R′ = $CO_2H$) was added to polyphosphoric acid at 160°, the reaction was complete in 3 min. and gave good yields of the oxide (III; R = OMe, R′ = $CO_2H$) which was readily reduced to the arsafluorene (V; R = OMe, R′ = $CO_2H$).

Optical resolution of 9-*p*-carboxyphenyl-2-methoxy-9-arsafluorene through the (+)- and (−)-1-phenylethylamine salts, and of 2-amino-9-phenyl-9-arsafluorene by (+)- and (−)-tartaric acids followed orthodox lines. In both cases difficulties were encountered in the separation of the diastereoisomeric salts in optically pure condition because solubility differences were small. In fact, effective resolution of the 9-*p*-carboxy-compound was impossible without an initial mechanical separation of the aggregates of prisms and fine needles which were deposited from solution almost simultaneously. The prisms proved to be the (+)-acid–(−)-base salt, which after crystallisation to constant rotation, $[\alpha]_D$ +110°, gave on decomposition (+)-acid $[\alpha]_D$ +156° (in pyridine). The rotation of the needles, (−)-acid–(−)-base salt, increased slowly to $[\alpha]_D$ −92°, but insufficient material was available for complete optical purification. This was obviously feasible, for the salts were optically stable: there was no evidence of second-order asymmetric transformation so frequently encountered with the corresponding antimony compounds. Using (+)-1-phenylethylamine with acid regenerated from intermediate fractions of salt gave (−)-acid–(+)-base salt, $[\alpha]_D$ −116°, from which (−)-acid, $[\alpha]_D$ −160·2° ± 1°, was obtained. This value made it clear that the (+)-acid, $[\alpha]_D$ +156°, was optically impure but residual (±)-acid was readily removed from it by one crystallisation from ethanol, and the pure (+)-acid had $[\alpha]_D$ +160·7° ± 1°.

Separation of the diastereoisomeric hydrogen (+)-tartrates of 2-amino-9-phenyl-9-arsafluorene was even more tedious, and again both antipodes of the resolving agent had to be used. However, this process gave the (+)-amine hydrogen (+)-tartrate, $[\alpha]_D$ +194°, from which (+)-amine, $[\alpha]_D$ +255° ± 1°, was isolated, and the corresponding hydrogen (−)-tartrate, $[\alpha]_D$ −193°, gave (−)-amine, $[\alpha]_D$ −251° ± 1°. In this case the small optical impurity could not be removed, for the (+)- and the (−)-amine separated as vitreous solids, m. p. 38—48° and 37—47°, respectively, and could not be crystallised. However, the enantiomeric acetyl derivatives, m. p. 182—184°, $[\alpha]_D^{20}$ +278·0° ± 1·2° and −279·5° ± 2·3°, were obtained optically pure. It should be noted that salt formation with tartaric acid of the same sign of rotation lowers the specific rotation of the (+)- and the (−)-amine, a feature also occurring with (+)- and (−)-2-amino-9-*p*-tolyl-9-stibiafluorenes.

The specific rotation of 9-*p*-carboxyphenyl-2-methoxy-9-arsafluorene, $[\alpha]_D$ +161°, is higher than that of the analogous stibiafluorene,[7] $[\alpha]_D$ +153°, but the calculated molecular rotations are $[M]_D$ +609° and +650° respectively. This indicates that here, as in the eutropic series of *iso*chromanium salts [12] containing asymmetric 3-covalent elements of Group VI the molecular rotation rises with increasing molecular weight of the element. Also, the optical stability of the compounds diminishes on going from arsenic to antimony, as it does with increasing molecular weight in the thio-, seleno- and telluro-*iso*chromanium salts. For instance, a solution of the (−)-arsafluorenecarboxylic acid in pyridine lost only 7·5% of its rotation after 30 days at room temperature, whereas a similar solution of the analogous antimony compound lost 50% of its activity in 41 hr. In fact, the arsafluorene showed remarkable optical stability, for, when a solution in chloroform containing 5% of ethanol (the compound is insufficiently soluble in either solvent alone) was heated in a sealed tube at 70°, the specific rotation, $[\alpha]_D$ −144°, fell barely 5%, to −137°, after 7 hours' heating. When the temperature was raised to 111°, however, the rotation dropped from $[\alpha]_D$ −143° to −13·3° in 1 hr., and, from the rate coefficients, the half-life was calculated as 17 min. It is possible, however, that the choice of chloroform–ethanol as solvent was unfortunate in this case, because of the possible generation of hydrogen chloride, a catalyst which was found to promote racemisation of the (+)-arsafluorene at room temperature. The catalytic effect of hydrogen chloride in this case was much less striking than in the stibiafluorene series. Unfortunately, racemisation data are not available for the antimony compound analogous to the arsafluorene under discussion, so

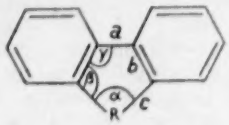
[12] Holliman and Mann, *J.*, 1945, 37.

direct comparison is not possible, but the half-life of (−)-2-carboxymethoxy-9-*p*-tolyl-9-stibiafluorene in chloroform–acetic acid containing a trace of hydrogen chloride at 26° was 5 min., whereas the half-life of 9-*p*-carboxyphenyl-2-methoxy-9-arsafluorene under similar conditions was 110 hr.

Solutions of (−)-2-amino-9-phenyl-9-arsafluorene in ethanol were optically stable at room temperature apparently indefinitely and no change in rotation was detected even after 1 hour's heating in a sealed tube at 110°. The amine, therefore, appears to be more, and the acid less, optically stable than predicted by Weston's calculations for a pyramidal molecule.[4] The half-life of the acid, 17 min. at 111°, is short compared with the value of 2 hr. at 107° calculated by Weston for the inversion (" racemisation ") of trimethylarsine, and the half-life of the (−)-amine is obviously considerably longer. Weston's model is, of course, aliphatic, and a similar assessment of the stability to inversion of an aromatic arsine would be of great interest.

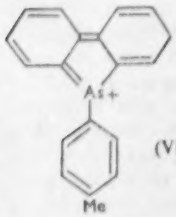
There seems little doubt that the arsafluorenes are, in fact, pyramidal molecules and evidence in support of this statement can be advanced from three sources. First, attempts to resolve 9-*p*-carboxyphenyl-9-arsafluorene have failed. This compound is symmetrical on a pyramidal model, but should be resolvable if the two benzene rings in the diphenyl residue are colinear but not coplanar.[11] Secondly, this " skew " configuration, suggested because it was considered that coplanarity of the tricyclic system could only be achieved at the expense of severe angular strain in the five-membered ring, appears unlikely in the light of geometrical calculations. Table 1 shows the probable dimensions of a 9-arsafluorene and the known dimensions of fluorene. The angles α, β, and γ were obtained by calculation using bond lengths of diphenyl obtained from electron-diffraction [13] and *X*-ray [14] measurements, and the length of the C–As bond as found in trimethylarsine.[15]

TABLE 1.




| | *a* (Å) | *b* (Å) | *c* (Å) | α | β | γ | Reference |
|---|---|---|---|---|---|---|---|
| R = As ..... | 1·54 | 1·39 | 1·98 | 96° | 102° | 120° | *a* & *b*, 13; *c*, 15. |
| R = As ..... | 1·48 | 1·42 | 1·98 | 94 | 103 | 120 | *a* & *b*, 14; *c*, 15. |
| R = C ..... | 1·486 | 1·410 | 1·468 | 105·6 | 109·6 | 107·6 | 16. |

These figures indicate that the total angular strain in the 5-membered ring of the heterocyclic molecule, in which the tricyclic system remains planar, is less than that occurring in the five-membered ring of fluorene, recently examined by *X*-rays [16] and shown to have a maximum deviation from the plane of 0·03 Å.




(VI)

Lastly, comparison of the ultraviolet spectra of 9-*p*-tolyl-9-arsafluorene [17] and triphenylarsine [18] shows that the main absorption band of the latter has undergone a bathochromic shift of 310 Å in the heterocyclic compound. This large displacement points to a considerable lowering of energy and indicates increased conjugation between the unshared pair of electrons on arsenic and the condensed ring system. The molecule, therefore, can best be represented by the numerous canonical forms of type (VI), and the whole tricyclic system in the arsafluorene must be essentially planar. The dissymmetry of the 9-arsafluorenes, therefore, arises from the disposition of the substituent in the 9-position above or below this plane, and the properties of the optically active 9-arsafluorenes show that the bonds from the arsenic atom can retain a stable pyramidal configuration.

[13] Karle and Brockway, *J. Amer. Chem. Soc.*, 1944, **66**, 1974.
[14] Dhar, *Indian J. Phys.*, 1932, **7**, 43.
[15] Springall and Brockway, *J. Amer. Chem. Soc.*, 1938, **60**, 996.
[16] Burns and Iball, *Nature*, 1954, **173**, 635.
[17] Campbell and Poller, *Chem. and Ind.*, 1953, 1126.
[18] Mann, Millar, and Smith, *J.*, 1953, 1130.

## Experimental

9-p-*Tolyl*-9-*arsafluorene*.—Diphenylyl-2-arsonic acid was prepared by the Bart reaction under conditions essentially those used by Cookson and Mann.[19] The maximum yield of the pure acid was only 27%, and this was obtained by addition of the diazonium salt solution under the surface of a large excess of aqueous sodium arsenite. After repeated crystallisation from 50% acetic acid, the compound had m. p. 216—222° (values obtained previously range from 202° to 206°), but analysis indicated that some anhydride had been formed. Cyclisation of this arsonic acid [9] by concentrated sulphuric acid at 90° gave 9-arsafluoreninic acid, m. p. 318—322° in 92% yield (m. p. 328° given in ref. 10), and this was converted into 9-chloro-9-arsafluorene, m. p. 161—162°, in 70% yield. A suspension of 9-chloro-9-arsafluorene (12·6 g.) in dry ether (45 ml.) was added slowly to *p*-tolylmagnesium bromide prepared from *p*-bromotoluene (24·8 g., 3 mols.) in ether (80 ml.), and the reaction mixture was boiled for 15 min. and filtered rapidly through glass wool. The product crystallised from this solution overnight, and recrystallisation from ethanol gave 9-p-*tolyl*-9-*arsafluorene* (V; R = H, R′ = Me), as colourless needles, m. p. 130·5—131·5° (70%) (Found: C, 71·3; H, 4·8; As, 23·5. $C_{19}H_{15}As$ requires C, 71·7; H, 4·8; As, 23·5%). From the ethereal mother-liquor a further 0·5 g. was isolated after decomposition of the excess of Grignard reagent. Attempts to characterise the arsafluorene by formation of dichloro- or dibromo-derivatives failed, because the initially crystalline dihalides were rapidly hydrolysed in moist air to the corresponding dihydroxy-compound. This also proved unsatisfactory as a derivative for it lost water at 114—130°, giving, presumably, an oxide (m. p. 206—207°). The mercurichloride, m. p. 219—222°, was deposited as colourless needles when hot ethanolic solutions of the constituents were mixed, and was found to be *bis*-9-p-*tolyl*-9-*arsafluorene trismercurichloride*, $(R_2As)_2(HgCl_2)_3$ (Found: C, 32·2; H, 2·3. $C_{38}H_{30}Cl_6As_2Hg_3$ requires C, 32·1; H, 2·1%).

9-p-*Carboxyphenyl*-9-*arsafluorene*.—When 9-*p*-tolyl-9-arsafluorene was oxidised by potassium permanganate under neutral conditions (with either magnesium sulphate or a phosphate buffer) the product obtained was largely the oxide containing little of the required carboxylic acid. The most successful oxidation occurred under alkaline conditions as follows. A mixture of crude 9-*p*-tolyl-9-arsafluorene, m. p. 127—130° (1·1 g.), potassium permanganate (2 g.), and sodium hydroxide (0·7 g.) in water (25 ml.) was boiled under reflux for 1¾ hr. The cooled mixture was decolorised by sulphur dioxide, and the product filtered off, washed, and dissolved in warm N-ammonia (40 ml.). Acidification of the filtered solution then gave 9-p-*carboxyphenyl*-9-*arsafluorene oxide* (III; R = H, R′ = $CO_2H$) (0·7 g.), m. p. 310—321° (decomp.), or m. p. 315—318° (decomp.) when inserted into the bath at 300° (Found: C, 62·2; H, 4·2. $C_{19}H_{13}O_3As$ requires C, 62·7; H, 3·6%). This oxide was reduced by the sulphur dioxide method,[6a] and the crude product was crystallised (twice) from ethanol. 9-p-*Carboxyphenyl*-9-*arsafluorene* (V; R = H, R′ = $CO_2H$) (0·4 g.) separated as colourless needles, m. p. 268—271° [Found: C, 65·5; H, 3·7; As, 21·5%; *M* (by titration in dimethylformamide with sodium methoxide), 346. $C_{19}H_{13}O_2As$ requires C, 65·5; H, 3·8; As, 21·5%; *M*, 348].

9-p-*Carboxyphenyl*-2-*methoxy*-9-*arsafluorene*.—*Method* 1. *Cyclisation of the arsonous chloride, followed by Grignard reaction.* 2-Amino-4′-methoxydiphenyl hydrochloride[20,7] (30·0 g.) in water (105 ml.) was treated with concentrated hydrochloric acid (17 ml.) and diazotised with sodium nitrite (9·6 g.) in water (18 ml.). The filtered solution was kept at 5° and added in portions under the surface of a stirred solution of sodium arsenite (33·0 g.), sodium carbonate (18·3 g.), and copper sulphate (0·6 g.) in water (165 ml.) at 60°. The tar which formed was filtered off and acidification of the filtrate gave 4′-*methoxydiphenylyl*-2-*arsonic acid*, m. p. 209—222° (12·8 g., 33%) (Found: C, 50·3; H, 4·2. $C_{13}H_{13}O_4As$ requires C, 50·7; H, 4·3%). Attempts to cyclise this acid by concentrated sulphuric acid resulted in sulphonation, so the acid was reduced by sulphur dioxide in the presence of concentrated hydrochloric acid and chloroform to 4′-methoxydiphenylyl-2-arsonous chloride, m. p. 63—67°. This crude chloride (7·7 g.) was heated at 200°, under reflux, for 2 hr. and the product dissolved in the minimum volume of boiling benzene. The solution, on cooling, deposited 9-*chloro*-2-*methoxy*-9-*arsafluorene* as yellow needles, m. p. 133—137° (3·1 g.), and a further fraction was obtained by evaporation of solvent and again heating to 200°. Recrystallisation from benzene gave the pure product, m. p. 136—137° (60%) (Found: C, 53·4; H, 3·4. $C_{13}H_{10}OClAs$ requires C, 53·4; H, 3·7%). A solution of 9-chloro-2-methoxy-9-arsafluorene (3·8 g., 1 mol.) in benzene (100 ml.)

[19] Cookson and Mann, *J.*, 1949, 2897.
[20] Copp and Walls, *J.*, 1950, 311.

was added to the Grignard reagent prepared from *p*-bromotoluene (8·9 g., 4 mols.) in ether (60 ml.). After boiling for 2·5 hr., the mixture was decomposed with 4N-hydrochloric acid, and the normal isolation procedure gave crude 2-methoxy-9-*p*-tolyl-9-arsafluorene (V; R = OMe, R′ = Me), m. p. 72—85° (5·3 g., 117% yield). Systematic crystallisation failed to remove the impurity which was almost certainly di-*p*-tolyl as steam-distillation gave a small fraction of arsenic-free material, m. p. 113—117°. Analyses of the compound (V; R = OMe, R′ = Me) were consistently poor (Found: C, 70·4; H, 4·8. Calc. for $C_{20}H_{17}OAs$: C, 69·0; H, 4·9%). Nevertheless, oxidation of the crude compound (0·5 g.) with alkaline potassium permanganate followed by reduction with sulphur dioxide gave 9-p-*carboxyphenyl-2-methoxy-9-arsafluorene* (V; R = OMe, R′ = $CO_2H$), m. p. 223° (0·3 g.) after crystallisation from ethanol (Found: C, 63·9; H, 4·4; As, 19·9. $C_{20}H_{15}O_3As$ requires C, 63·5; H, 4·0; As, 19·8%). An arsenic-free impurity, m. p. >340°, insoluble in ethanol, was also isolated, gave the characteristic smell of diphenyl on decarboxylation, and was probably 4 : 4′-di-*p*-carboxydiphenyl. This confirmed di-*p*-tolyl as the impurity in the tolyl compound.

*Method* 2. *Cyclisation of* (IV; R = OMe, R′ = $CO_2H$). *p*-Aminobenzoic acid (50·0 g.) was converted into *p*-carboxyphenylarsonic acid, m. p. >360° (44·3 g.), by the method of Lewis and Hamilton,[21] and this was reduced by sulphur dioxide to *p*-carboxyphenylarsonous chloride, m. p. 156—162° (31·2 g.). 2-Amino-4′-methoxydiphenyl hydrochloride (5·9 g.) in absolute ethanol (25 ml.) was treated with a saturated ethanolic solution of hydrogen chloride (6·5 ml.), cooled to 5°, and diazotised with pentyl nitrite (4 ml.) in ethanol (10 ml.). The resulting solution, at 5°, was added in portions to *p*-carboxyphenylarsonous chloride (6·0 g.), in ethanol (60 ml.) which was kept at 40—50° and vigorously stirred. Copper bronze (0·4 g.) was added during this addition. When the evolution of nitrogen ceased, ethanol was removed under reduced pressure and the crude arsinic chloride was hydrolysed by warm 4N-sodium hydroxide (30 ml.). After cooling, ether was added to dissolve arsenic-free by-products, and the mixture was acidified to Congo-red with 4N-hydrochloric acid, the crude arsinic acid being precipitated. Purification by reprecipitation from solution in sodium hydrogen carbonate gave p-*carboxyphenyl-2-4′-methoxydiphenylylarsinic acid* (II; R = OMe, R′ = $CO_2H$), m. p. 271—278° (decomp.) (corr.) (3·8 g., 41%). This material was used in subsequent reactions without further purification, but one crystallisation from 50% aqueous acetic acid raised the m. p. to 280—281° (decomp.) (corr.) (Found: C, 58·8; H, 4·3. $C_{20}H_{17}O_5As$ requires C, 58·3; H, 4·2%). The arsinic acid was reduced by sulphur dioxide to p-*carboxyphenyl-2-4′-methoxydiphenylylarsinous chloride* (IV; R = OMe, R′ = $CO_2H$), m. p. 200—204° (55%) (Found: C, 57·8; H, 4·0. $C_{20}H_{16}O_3ClAs$ requires C, 57·9; H, 3·9%). Cyclisation of this chloride (1·5 g.) by heating at 250°/14 mm. for 1 hr. gave a white sublimate, m. p. 86—89°, found to be 4-methoxydiphenyl, and a vitreous solid from which boiling ethanol extracted the compound (V; R = OMe, R′ = $CO_2H$), m. p. 218—222° (0·4 g.).

*Method* 3. *Cyclisation of* (II; R = OMe, R′ = $CO_2H$), *followed by reduction.* The polyphosphoric acid [22] used in this ring closure was prepared by dissolving phosphoric oxide (24 g.) in syrupy phosphoric acid (*d* 1·75; 20 ml.). When the acid (II; R = OMe, R′ = $CO_2H$) (1 g.) was added to polyphosphoric acid (5 g.) at 160°, the temperature of the stirred mixture dropped 10° and rose again. After 3 min. at 160°, the homogeneous solution was poured into water, and the precipitated solid was filtered off and dried. This solid, m. p. 296—302° (decomp., corr.) (0·9 g.), which was the oxide (III; R = OMe, R′ = $CO_2H$), was reduced by sulphur dioxide, and crystallisation of the product from ethanol gave the acid (V; R = OMe, R′ = $CO_2H$), m. p. 223—224° (0·7 g., 76%), unchanged on admixture with a sample made by method 1 or 2. This ring closure proceeded well with 5 g. of the arsinic acid, though the yield was somewhat lower.

*2-Amino-9-phenyl-9-arsafluorene.—Preparation of 2-4′-nitrodiphenylylphenylarsinic acid. Method* 1. *Under aqueous alkaline conditions.* This reaction was carried out under conditions essentially those used by Blicke and Webster [23] for the preparation of *m*-nitrodiphenylarsinic acid. A solution of phenylarsonous chloride (6 g.) in sodium hydroxide (4·3 g. in 30 ml. water) was slowly added to the neutralised diazonium salt solution obtained from 2-amino-4′-nitrodiphenyl [24] (5·8 g.). The recommended method of isolation failed in this case, but on neutralisation of the mixture with 4N-acetic acid a dark brown solid was obtained. Extraction of this with 4N-sodium hydroxide followed by acidification gave 4′-*nitro-2-diphenylylphenylarsinic acid* (II; R = $NO_2$, R′ = H), m. p. 240—243° (0·6 g.) after crystallisation from ethanol from which

[21] Lewis and Hamilton, *J. Amer. Chem. Soc.*, 1923, **45**, 758.
[22] Evans and Smith, *J.*, 1954, 798.
[23] Blicke and Webster, *J. Amer. Chem. Soc.*, 1937, **59**, 534.
[24] Scarborough and Waters, *J.*, 1927, 89.

it separated as yellow needles (Found: C, 56·2; H, 4·2. $C_{18}H_{14}O_4NAs$ requires C, 56·4; H, 3·7%). In some experiments, particularly if the neutralised diazonium and phenylarsonous oxide solutions were mixed rapidly, the main product was di-(4′-nitro-2-diphenylyl)arsinic acid, m. p. 295—299° (decomp.) (in ref. 9 the m. p. is quoted as >260°) (Found: C, 57·1; H, 4·2. Calc. for $C_{24}H_{17}O_6N_2As$: C, 57·3; H, 3·4%).

*Method* 2. *Reaction in absolute ethanol under acid conditions.* 2-Amino-4′-nitrodiphenyl (5·4 g.) in absolute ethanol (50 ml.) was treated with saturated ethanolic hydrogen chloride (8 ml.) and diazotised at 5° with pentyl nitrite (4 ml.) in absolute ethanol (10 ml.). The resulting solution was added dropwise to one of phenylarsonous chloride (5·6 g.) in ethanol (50 ml.) kept at 60° and vigorously stirred. At each addition a transient yellow precipitate appeared and decomposed with evolution of nitrogen. Copper bronze (0·2 g.) was added to increase the rate of decomposition. The crude product was isolated from this solution by the method used for the acid (II; R = OMe, R′ = $CO_2H$) and had m. p. 216—236° (decomp.) (6·7 g.). Crystallisation from ethanol or from 50% acetic acid gave the product (II; R = $NO_2$, R′ = H), m. p. 241—244° (3·1 g., 32%) unchanged on admixture with a sample obtained by method 1.

*2-Nitro-9-phenyl-9-arsafluorene 9-oxide.* The above arsinic acid (2·5 g.) was cyclised by warming it in concentrated sulphuric acid (30 ml.) at 140—150° for 1 hr. The product was isolated by pouring the cooled solution on cracked ice, and crystallisation of the precipitate from 50% acetic acid (12 ml.) gave *2-nitro-9-phenyl-9-arsafluorene 9-oxide* (III; R = $NO_2$, R′ = H) (2·1 g.) as buff leaflets, which melted at 120°, resolidified on further heating, and remelted at 272—273° (Found: C, 59·7; H, 3·3; N, 3·8. $C_{18}H_{12}O_3NAs$ requires C, 59·2; H, 3·7; N, 4·0%).

*2-Amino-9-phenyl-9-arsafluorene.* Simultaneous reduction of the nitro-group and removal of oxygen occurred when the above oxide (5 g.) was heated with stannous chloride (17·5 g.), concentrated hydrochloric acid (17·5 ml.), and ethanol (25 ml.) under reflux for 2 hr. At 0° the solution gradually deposited the stannichloride of the base, which was isolated and decomposed with 4N-sodium hydroxide. Extraction of the amine with ether gave a glass which was converted into the *hydrochloride* (V; R = $NH_3Cl$, R′ = H), colourless needles, m. p. 220—224° (decomp.) (3·4 g., 69%), after crystallisation from carbon tetrachloride (Found: C, 60·5; H, 4·3. $C_{18}H_{15}NClAs$ requires C, 60·8; H, 4·3%). Base regenerated from this hydrochloride again formed a glass but a specimen regained from the (+)-tartrate [once recrystallised and consequently probably slightly enriched with (+)-base] gave a satisfactory analysis (Found: C, 68·1; H, 4·7; N, 4·0. $C_{18}H_{14}NAs$ requires C, 67·7; H, 4·4; N, 4·4%). The *acetyl derivative* (V; R = NHAc, R′ = H) had m. p. 156—157° (Found: C, 66·0; H, 4·6. $C_{20}H_{16}ONAs$ requires C, 66·5; H, 4·5%).

*Attempted Resolution of 9-p-Carboxyphenyl-9-arsafluorene.*—Specific rotations were measured in absolute ethanol in 2 dm. tubes unless otherwise stated.

(1) The acid (1 g.) was dissolved in boiling ethanol (110 ml.) containing (−)-1-phenylethylamine (0·7 g., 2 equivs.) and, on cooling, deposited a first fraction of salt, F1, m. p. 200—210°, $[\alpha]_D$ −6·7° ± 2° (*c* 0·255) (0·85 g.). Concentration of the mother-liquor to half volume gave a second fraction, F2, m. p. 203—208°, $[\alpha]_D$ −6·8° ± 2° (*c* 0·265) (0·35 g.). Decomposition of F2 with 0·1N-sulphuric acid at −10° gave an acid, m. p. 263—268°, which was optically inactive when examined in pyridine. F1 was recrystallised three times from ethanol, and the final *salt* (0·3 g.) had m. p. 208—210°, $[\alpha]_D$ −9·5° ± 2° (*c* 0·263) (Found: C, 69·3; H, 5·4. $C_{37}H_{34}O_3NAs$ requires C, 69·1; H, 5·2%). This increase in specific rotation was evidently caused by chemical purification for decomposition gave a specimen of acid, m. p. 268—269°, which was optically inactive.

(2) The *quinine salt*, m. p. 207—208°, $[\alpha]_D$ −87·8° ± 1° (*c* 0·458), separated in 90% yield when the acid was mixed with twice the calculated quantity of quinine in boiling ethanol (Found: C, 69·8; H, 5·8. $C_{39}H_{37}O_4N_2As$ requires C, 69·6; H, 5·5%). This salt was recrystallised five times from ethanol and showed no change in specific rotation. The final fraction, m. p. 207—208°, had $[\alpha]_D$ −86·8° ± 2·2° (*c* 0·255), and acid regained from it showed no rotation when examined in pyridine.

*Resolution of 9-p-Carboxyphenyl-2-methoxy-9-arsafluorene.*—The acid (6·5 g.) was added to (−)-1-phenylethylamine (4·16 g., 2 equivs.) in boiling ethanol (530 ml.), and the filtered solution set aside at room temperature. During the slow crystallisation, a layer of elongated prisms separated initially and clumps of fine needles were deposited above it; both forms then grew simultaneously. After 66 hr. the mother-liquor was decanted, and the crystals were separated mechanically into prisms, A1, and needles, A2. The decanted mother-liquor gave fraction A3 when kept for 48 hr., and successive fractions were obtained by concentration under reduced

pressure (see Table 2). A1 was crystallised four times from ethanol containing a little (−)-1-phenylethylamine, and the apparently pure (+)-*acid*–(−)-*base salt* obtained had m. p. 224°, $[\alpha]_D$ +110° ± 2° (*c* 0·216) (0·69 g.) (Found: C, 67·1; H, 5·2. $C_{28}H_{26}O_3NAs$ requires C, 67·3; H, 5·2%). Fractions A2, A3, and A4 were combined and recrystallised six times from ethanol but

TABLE 2.

| Fraction | Wt. (g.) | $[\alpha]_D$ | *c* (EtOH) | M. p. | Fraction | Wt. (g.) | $[\alpha]_D$ | *c* (EtOH) | M. p. |
|---|---|---|---|---|---|---|---|---|---|
| A1 | 2·75 | +30·0° | 0·220 | 200—220° | A4 | 1·30 | −45·8 | 0·207 | — |
| A2 | 1·23 | −50·6 | 0·240 | 197—200 | A5 | 1·32 | −12·4 | 0·237 | 195—202 |
| A3 | 0·55 | −60·7 | 0·224 | 195—202 | A6 | 0·29 | 0 | 0·218 | 192—210 |

the most lævorotatory fraction, m. p. 206—207°, $[\alpha]_D$ −92° (*c* 0·206) (0·2 g.), had not reached optical purity. Crystallisation from ethyl acetate or *iso*propyl alcohol also failed to yield the pure (−)-acid–(−)-base salt and consequently the acid was regenerated from all intermediate fractions. This acid (3·5 g.) was treated with (+)-1-phenylethylamine (2·24 g., 2 equivs.) in hot ethanol (350 ml.). Again the diastereoisomeric salts differed characteristically in crystalline form and were separated mechanically. The prisms, $[\alpha]_D$ −68·4° (*c* 0·223) (1·4 g.), after three recrystallisations from ethanol containing a little free (+)-base gave the optically pure (−)-*acid*–(+)-*base salt*, m. p. 225—226°, $[\alpha]_D$ −116° ± 2° (*c* 0·215) (0·48 g.) (Found: C, 67·0; H, 5·3%).

*Isolation of the* (+)- *and the* (−)-*acid.* Addition of excess of 0·1N-sulphuric acid to a solution of the appropriate salt, dissolved in ethanol and kept at −10°, precipitated the crystalline active acid. Decomposition showed that the (+)-acid–(−)-base salt had not reached optical purity, but complete optical purification was effected when the (+)-acid liberated was crystallised once from ethanol. Details of the active acids are given in Table 3.

TABLE 3.

| Resolving agent | Salt, $[\alpha]_D$ | Acid, $[\alpha]_D^{21}$ | Acid, $[\alpha]_{5461}$ | *c* in pyridine | M. p. | Found (%) in acid C | H |
|---|---|---|---|---|---|---|---|
| (−)-CHPhMe·$NH_2$ ... | +110° | +156° | — | 0·494 | 239—240° | — | — |
| (−)-CHPhMe·$NH_2$ ... | −92 | −111 | — | 0·206 | 230—233 | — | — |
| (+)-CHPhMe·$NH_2$ ... | −116 | −160 | −198 | 0·476 | 240 | 63·2 | 4·3 |
| | | +161 * | +196 | 0·488 | 240 | 63·0 | 4·0 |

* (+)-Acid, $[\alpha]_D$ +156°, recrystallised from ethanol.

*Resolution of* 2-*Amino*-9-*phenyl*-9-*arsafluorene.*—The amine, regenerated from the hydrochloride (7·1 g.) by addition of N-sodium hydroxide, was added to (+)-tartaric acid (3·3 g., 1·1 mol.) dissolved in boiling ethanol (120 ml.), and the solution rapidly filtered. The salt (8·35 g., 88%) which separated overnight had m. p. 158—160° (decomp.), $[\alpha]_D$ +4·0°. Rotations were measured in "Analar" acetone (*c* ~ 0·25). Concentration of the mother-liquor gave a small, rather discoloured second fraction, $[\alpha]_D$ −26·3°. The first fraction was recrystallised eight times from ethanol containing 1% of (+)-tartaric acid and then had m. p. 158—160° (decomp.), $[\alpha]_D$ +28·9° (4·56 g.). Resolution was obviously very slow, consequently a different solvent for crystallisation seemed advisable, despite the fact that previous small-scale experiments had indicated that the (+)-base (+)-tartrate could be obtained in essentially optically pure condition by using ethanol. Two recrystallisations from methanol (4 ml. per g. of salt) gave a fraction having $[\alpha]_D$ +51·0° (3·28 g.), and four further crystallisations from the same solvent gave pure (+)-*base* (+)-*tartrate*, as pale buff plates, m. p. 159—161° (decomp.), $[\alpha]_D$ +194° ± 2° (0·42 g.) (Found: C, 56·1; H, 4·6. $C_{32}H_{30}O_6NAs$ requires C, 56·3; H, 4·3%). In crystallisations from methanol it was found essential to prevent evaporation of the solvent or little increase in $[\alpha]_D$ occurred. Recrystallisation of lævorotatory fractions indicated that isolation of (−)-base (+)-tartrate should be possible but tedious. Consequently all intermediate fractions were decomposed, giving impure (−)-base, $[\alpha]_D$ −47·5°. The fraction of salt (3·3 g.) obtained from this base (3·5 g.) with (−)-tartaric acid (1·8 g., 1·1 mol.) in ethanol (70 ml.) had m. p. 152—155° (decomp.), $[\alpha]_D$ −47·3°. One crystallisation of this from methanol raised the rotation of the salt (1·83 g.) to $[\alpha]_D$ −121° and three further crystallisations from ethanol gave the (−)-*base* (−)-*tartrate*, m. p. 158—159° (decomp.), $[\alpha]_D$ −193° ± 2° (0·38 g.) (Found: C, 56·2; H, 4·7%).

*Isolation of the* (+)- *and the* (−)-*amine.* The appropriate salt was dissolved in the minimum volume of methanol, and 0·1N-sodium hydroxide was added at −10°. In this way the (+)-amine was precipitated as a colourless gum which solidified to a pale brown glass, m. p. 38—48°

(hot-stage microscope), $[\alpha]_D^{25}$ +255° ± 1°, $[\alpha]_{5461}^{25}$ +321° ± 1°, and the (−)-amine had m. p. 37—47°, $[\alpha]_D^{25}$ −251° ± 1°, $[\alpha]_{5461}^{25}$ −318° ± 1° (*c* 0·45 in EtOH) (Found: C, 67·0; H, 4·5. $C_{18}H_{14}NAs$ requires C, 67·7; H, 4·4%). Acetic anhydride at room temperature gave the corresponding (+)- and (−)-*acetyl derivative*, m. p. 187—189°, $[\alpha]_D^{20}$ +278·0° ± 1·2° (*c*, 0·205 in EtOH), and $[\alpha]_D$ −279·5° ± 2·3° (*c* 0·110 in EtOH) (Found: C, 66·1; H, 4·7. $C_{20}H_{14}ONAs$ requires C, 66·5; H, 4·5%).

*Racemisation.*—The results of racemisation experiments on the (−)-acid (V; R = OMe, R′ = $CO_2H$) are given in Table 4. "AnalaR" chloroform containing 5% of ethanol was used

TABLE 4.

| Solvent | *c* | Temp. | Initial $[\alpha]_D$ | Final $[\alpha]_D$ | Time | $10^3k$ |
|---|---|---|---|---|---|---|
| Pyridine | 0·467 | 20—22° | −160° | −160° | 6 days | — |
| $CHCl_3$–EtOH | 0·254 | 70 | −144 | −137 | 7 hr. | — |
| $CHCl_3$–EtOH | 0·251 | 111 | −143 | −42·4 | 30 min. | 4·1 |
| $CHCl_3$–EtOH | 0·251 | 111 | −143 | −13·3 | 60 min. | 4·0 |

$k = (\ln \alpha_0/\alpha_t)/t$ in min.$^{-1}$.

as solvent, because it was impossible to dissolve 50 mg. of the (−)-acid in 20 ml. of any of the common neutral organic solvents. Solutions were sealed in glass bulbs which were heated in a thermostat for the periods stated. The bulb was then cooled rapidly and the solution examined polarimetrically at 20°. Acid regained from the solution with $[\alpha]_D$ −13·3° had m. p. 219—221° and had m. p. 221—224° when mixed with (±)-acid. The catalytic effect of hydrogen chloride was demonstrated when a glass rod which had been in contact with ethanolic hydrogen chloride was dipped into a solution of the (+)-acid. The initial rotation, $\alpha_D$ +0·72°, fell to $\alpha_D$ +0·36 in 110 hr. and reached zero after about 500 hr. at room temperature. This racemisation did not follow the unimolecular rate law.

Solutions of the (+)-amine (V; R = $NH_2$, R′ = H) in ethanol were heated in sealed bulbs at 110°. No change in rotation was detected after 15 or 60 min.

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## 251. *The Allomerization of Chlorophyll.*

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The autoxidation of chlorophyll in methanol ("allomerization") and in other solvents is influenced by the solvent, the concentration of the chlorophyll, and dissolved salts. The absorption spectra of the products are reported and the mechanism of allomerization is briefly discussed.

"ALLOMERIZATION" is the term applied to the autoxidation of chlorophyll in alcoholic solution which precludes it from showing a positive Molisch phase test, the transient brown coloration on addition of alcoholic potassium hydroxide. Allomerization is associated with an uptake of oxygen equimolar with the chlorophyll present.[1] Conditions for promotion of the reaction have not previously been systematically investigated, except for evidence that the alcoholic solvent plays a part.[2] The reaction has been envisaged as the formation of an unstable hydroperoxide on $C_{(10)}$ (of Fischer's formula), and its conversion into an alkyl ether by the solvent.[2]

### EXPERIMENTAL

Chlorophyll-*a* and -*b* were prepared from fresh spinach leaves by Zscheile and Comar's method[3] except that 0·5% propan-2-ol in light petroleum was used for chromatographic

[1] Conant, Bailey, and Kammerling, *J. Amer. Chem. Soc.*, 1931, **53**, 2838.
[2] Fischer and Strell, "FIAT Review of German Science. Biochemistry, Part 1," 1947, p. 141.
[3] Zscheile and Comar, *Bot. Gaz.*, 1941, **102**, 463.

separation.[4] The pigments were re-chromatographed to attain the accepted spectral standards of purity[5] and were stored at 0° in purified ether.

Methanol was dried by refluxing it with sodium and dimethyl phthalate and distilling it.[6] Drying by magnesium turnings activated by iodine, and distillation without a fractionating column, yielded a solvent in which chlorophyll rapidly allomerized; the acceleration was apparently caused by traces of magnesium methoxide. A stock of such a solvent provided a convenient method for preparing the allomerized product.

Anhydrous halides were of the purest grades commercially available and did not require drying since up to 1% of water had no detectable influence on the reaction. A decline in allomerizing activity occurred on storage of stock solutions of halides in methanol (Table 1), perhaps by conversion of halide into less reactive methoxide. The effect of this complication was minimized by preparation of stock salt solutions just before use.

TABLE 1. *Accelerating activity of fresh and 24 hr.-old solutions of halides in* $6{\cdot}6 \times 10^{-6}$M-*solutions of chlorophyll-*a *in methanol.*

| Salt | Concn. ($10^{-6}$ M) | Allomerization (%) after 3 hr. for : fresh solns. | 24 hr.-old solns. | Salt | Concn. ($10^{-6}$M) | Allomerization (%) after 3 hr. for : fresh solns. | 24 hr.-old solns. |
|---|---|---|---|---|---|---|---|
| $LaCl_3$ ... | 0·61 | 92 | 76 | $MgCl_2$ ... | 11·5 | 67 | 43 |
| | 0·57 | 40 | 24 | | 7·4 | 43 | 23 |
| | 0·41 | 12 | 9 | | 4·9 | 30 | 14 |

Ethoxides and methoxides were prepared by direct reaction of metal and alcohol, followed by vacuum-distillation and recrystallization.

The fluorimeter and fluorescence cells have been described previously.[7] A Wratten No. 88 filter effectively cut off radiation of less than 7100 Å from the photocell. Absorption spectra were measured by Beckman and Unicam photoelectric spectrophotometers.

Extents of allomerization of dilute solutions of chlorophyll-*a* were obtained from absorption measurements at 4200 and 3900 Å. Wavelength 4200 Å was chosen since a large change of absorption occurred there during allomerization. Conversely, 3900 Å is a crossing-point of the spectra of reactant and product, no significant change in absorption during allomerization being shown by the following typical data for a $5{\cdot}6 \times 10^{-6}$M-solution of chlorophyll-*a* in methanol :

| | | | | |
|---|---|---|---|---|
| Allomerization (%) ..... | 5 | 10 | 68 | 98 |
| $(\log I_0/I)_{4200}$ ..... | 0·337 | 0·340 | 0·345 | 0·340 |

From the two measurements at each extent of allomerization, the fraction of chlorophyll remaining, $a/a_0$, was obtained from :

$$a/a_0 = (s - s_X)/(s_A - s_X) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where $s$, $s_A$, and $s_X$ are the ratios of absorption at 4200 and 3900 Å observed for the mixture, for chlorophyll-*a*, and for the allomer, respectively. Since the measurement was independent of dilution and different fillings of the photometer cells, errors from these causes were eliminated. From 10% to 90% of allomerization the error in the calculation of the fraction of chlorophyll remaining was ±1%, and outside this region it was ±2%.

Extents of allomerization of more concentrated solutions of chlorophyll-*a* were deduced from similar spectral ratios, $s$ (see p. 1209). With the concentrated solutions also, the absorption at 3900 Å remained almost unchanged during allomerization, as illustrated by the following measurements :

| | | | | |
|---|---|---|---|---|
| Chlorophyll-*a* concn. ($10^{-4}$ mole/l.) ..... | 0·8 | 3·2 | 8·0 | 32·0 |
| $(\log I_0/I)_{3900}$ initially ..... | 0·429 | 0·422 | 0·438 | 0·465 |
| $(\log I_0/I)_{3900}$ after complete allomn. ..... | 0·420 | 0·428 | 0·458 | 0·460 |

## RESULTS

*Dilute Chlorophyll Solutions.*—It is found that allomerization to give one product is predominant below $10^{-4}$M-chlorophyll concentration. Reaction giving two more products becomes important at higher concentrations. For this reason, it is convenient to designate chlorophyll concentrations less than $10^{-4}$M as the dilute solution range.

[4] Strain, *Ind. Eng. Chem. Anal.*, 1946, **18**, 605.
[5] Comar and Zscheile, *Plant Physiol.*, 1942, **17**, 198.
[6] Livingston and Pariser, *J. Amer. Chem. Soc.*, 1948, **70**, 1510.
[7] Livingston, Watson, and McArdle, *ibid.*, 1949, **71**, 1542.

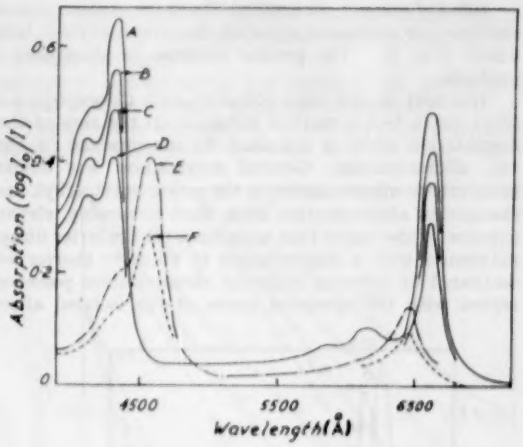



FIG. 1. *Decomposition of chlorophyll-a and -b in acetone.*

—— Chlorophyll-*a*; – – – chlorophyll-*b*.

*A*, 1 hr. at 25°.
*B*, 24 hr. at 56°.
*C*, 48 hr. at 56°.
*D*, 120 hr. at 56°.
*E*, 14 hr. at 25°.
*F*, 64 hr. at 56°.

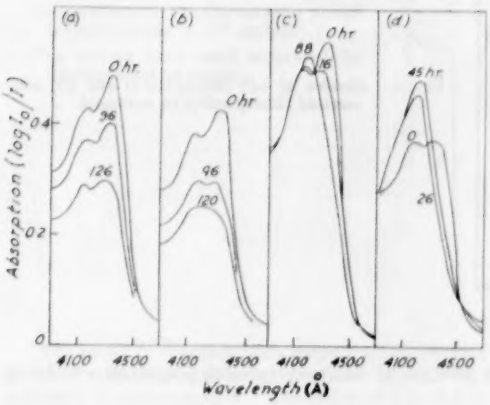



FIG. 2. *Decomposition and allomerization of chlorophyll-a in (a) octanol, (b) n-butanol, (c) ethanol, and (d) methanol at 65°.*

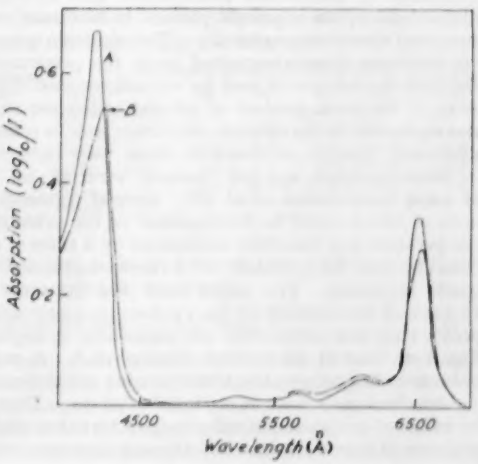



FIG. 3. *Spectra of allomerized chlorophyll-a in (A) ether and (B) in methanol and ethanol.*

*Effect of solvent.* In acetone, the shape of absorption spectra of chlorophyll-*a* and -*b* remained unaltered on prolonged exposure to oxygen at room temperature and when the solvent was refluxed (Fig. 1). The general decrease in absorption demonstrates the absence of coloured products.

Different results were obtained with chlorophyll-*a* in alcoholic solvents. The size of the alkyl group had a marked influence on the rate of the competitive processes, (i) the general degradation noted in non-alcoholic solvents and (ii) the specific change occurring in alcohols, *viz.*, allomerization. General degradation was dominant in octyl alcohol, but decreased relatively to allomerization in the series, butyl, ethyl, and methyl alcohol (Fig. 2). The spectral changes on allomerization were, most noticeably, elevation of the minor blue maximum at the expense of the major blue maximum till the latter disappeared, and a slight decrease of the red maximum with a displacement of 80 Å to shorter wavelength. The absorption spectra in methanol at different stages of allomerization possessed six intersection points in the visible region with the spectrum curve of the isolated allomerized product, showing that general

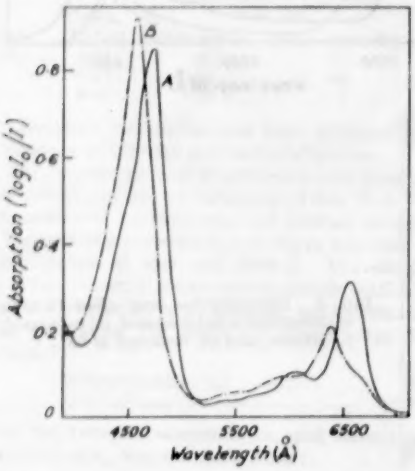



FIG. 4. *Spectra of (A) chlorophyll-*b *and (B) allomerized chlorophyll-*b *in methanol.*

degradation could be neglected and that a product of constant spectral properties was being produced.

*Spectra of allomerized chlorophylls- and chlorophyllides-*a *and* -b. After several weeks' allomerization, the pigments present in methanol and ethanol solutions of chlorophyll-*a* were separated chromatographically. The alcoholic solvents were replaced by light petroleum, and the solutions chromatographed as in the preparation of chlorophyll. One major band was obtained in addition to that for non-allomerized chlorophyll. The spectra in methanol and in ether of the main product of allomerization are recorded in Fig. 3. Two minor components also separated on the column, of interest later in considering allomerization in more concentrated solutions. Spectra of identical shape were obtained on allomerizing ethyl chlorophyllide-*a*.

More complex spectral changes occurred in alcoholic solutions of chlorophyll-*b* kept at room temperature or at 65°. Several pigments separated on a chromatographic column, none of which could be distinguished as the primary coloured product. The reaction at room temperature was therefore accelerated by a trace of magnesium chloride and also by lanthanum chloride, and the products were chromatographed within 24 hr. One major and two minor bands separated. The major band was the same whichever accelerator was used (Fig. 4). In view of the identity of the product on acceleration of chlorophyll-*a* by these salts with the product on non-accelerated allomerization, it appears justifiable to consider the spectrum of Fig. 4 as that of allomerized chlorophyll-*b*. A main product with this spectrum was also obtained after accelerated allomerization of ethyl chlorophyllide-*b*.

*Acceleration by salts in methanol solutions.* The rate of allomerization was greatly increased by traces of certain metal salts, to give for chlorophyll-*a* a product stable for several days in the presence of excess of salt. No allomerization occurred on mixing rigorously degassed solutions of

chlorophyll and salt in an H-tube. However, allomerization appeared relatively insensitive to oxygen concentration, since evacuation to *ca.* 1 mm. immediately after mixing retarded the rate of accelerated allomerization only to about one-third.

First-order rate dependence on chlorophyll concentration was observed for accelerated allomerization (see Fig. 5). Relative values of apparent rate constants at equimolecular salt concentrations are recorded in Table 2. The nature of the cation is clearly the most important factor, although a small effect of the anion can be observed.

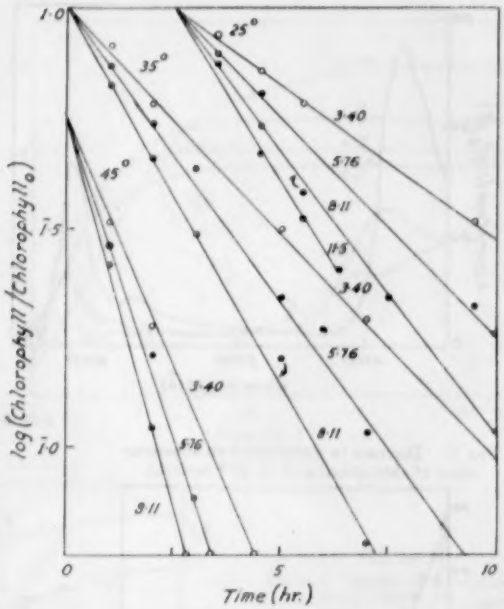



FIG. 5. *Acceleration of allomerization of chlorophyll-a by magnesium chloride.*
Chlorophyll concn., $6{\cdot}6 \times 10^{-6}$ mole/l.
The numbers on the curves refer to salt concentrations $\times 10^{3}$ mole/l.
(The curves have been separated by displacement of origin.)

The order of reaction with respect to accelerator concentration [equation (2) being assumed to hold] was obtained for magnesium chloride by varying salt concentration in $6{\cdot}6 \times 10^{-6}$M-solutions of chlorophyll-*a*. The slopes of Fig. 5 give $k'$, from which $a$ and $k$ have values at 25°, 35°, and 45° of 0·65, 0·65, and 0·65, and $3{\cdot}63 \times 10^{-3}$, $5{\cdot}08 \times 10^{-3}$, and $1{\cdot}06 \times 10^{-1}$ mole$^{-0{\cdot}65}$ l.$^{0{\cdot}65}$ sec.$^{-1}$ respectively. The calculated activation energy is 10 kcal./mole.

TABLE 2. *Effects of salts on the allomerization of chlorophyll-a in methanol.*

Concns. (mole/l.): chlorophyll-*a*, $6{\cdot}4 \times 10^{-6}$; salt, $6{\cdot}0 \times 10^{-3}$.

| Salt | Allomerization (%) after (hr.) 1 | 3½ | 6 | 8 | 27 | Relative rates |
|---|---|---|---|---|---|---|
| None | — | — | 1·0 | 1·2 | 6·7 | 1 |
| NaCl | — | 1·2 | 2·4 | — | 10·6 | 1·6 |
| NaBr | — | 1·2 | 1·6 | — | 15·9 | 2·4 |
| NaI | — | 1·2 | — | — | 17·6 | 2·6 |
| CsBr | — | 2·0 | 2·3 | 2·7 | 8·2 | 1·2 |
| CsI | — | 1·0 | 1·2 | 2·0 | 11·8 | 1·8 |
| $MgCl_2$ | 43 | 86·0 | 96·8 | 100 | 100 | 99 |
| $MgBr_2$ | 33 | 80·0 | 96·5 | 100 | 100 | 92 |
| $MgI_2$ | 37 | 84·7 | 92·2 | 97·0 | 100 | 98 |
| $BaCl_2$ | — | 2·0 | 4·3 | 7·5 | 32·2 | 4·8 |
| $BaBr_2$ | — | 2·0 | 7·1 | 8·2 | 35·2 | 5·2 |
| $BaI_2$ | — | 3·9 | 11·8 | 17·6 | 50·2 | 7·5 |
| $LaCl_3$ | 100 | — | — | — | — | >40 |

$$-\mathrm{d[Chlor.]}/\mathrm{d}t = k[\mathrm{Accel.}]^{a}[\mathrm{Chlor.}] = k'[\mathrm{Chlor.}] \quad . \quad . \quad . \quad . \quad (2)$$

Similar experiments with lanthanum chloride gave no realistic value for order of reaction, there being a narrow range of catalyst concentration over which the rate changed from very slow to too rapid to be measured spectrophotometrically. The lower limit was reached with lanthanum chloride concentrations of the order of the chlorophyll concentration.

*Acceleration by salts in ethereal solutions.* Addition of salts to ethereal solutions produced

more extensive changes in absorption spectra of chlorophyll-*a* (Fig. 6). Methanol was required for dissolution of the salt, and also appeared to have a protective action against extensive decomposition. Visually, the solutions became straw-yellow and non-fluorescent. The change was not reversed by replacing the ether by methanol in absence of air throughout the operation.

*Fluorescence changes.* The measured fluorescence intensity decreased on allomerization of chlorophyll-*a*. For $5 \times 10^{-6}$M-solutions in methanol, the intensity decreased to a constant value of 55% of the original in absence of added catalyst, and with various concentrations of lanthanum

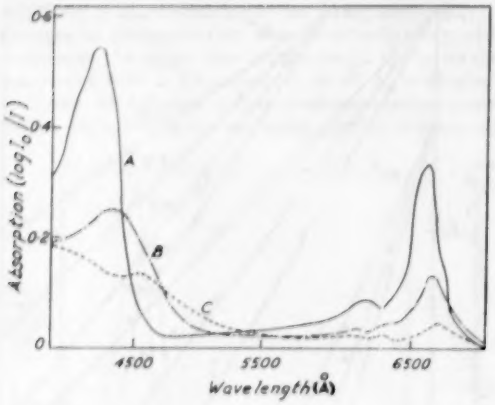


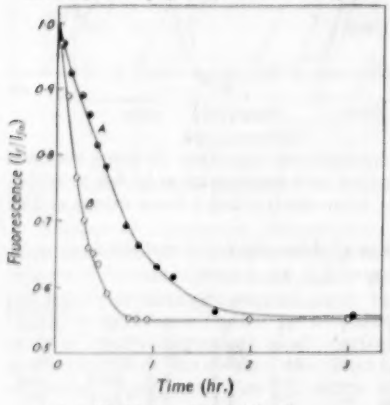

FIG. 6. *Spectra 24 hr. after addition of lanthanum chloride to ether–methanol solutions of chlorophyll-a* ($5 \times 10^{-6}$ mole/l.).

*A*, in 10% methanol–ether.

*B*, in 20% methanol–ether, with $10^{-3}$ mole/l. of $LaCl_3$.

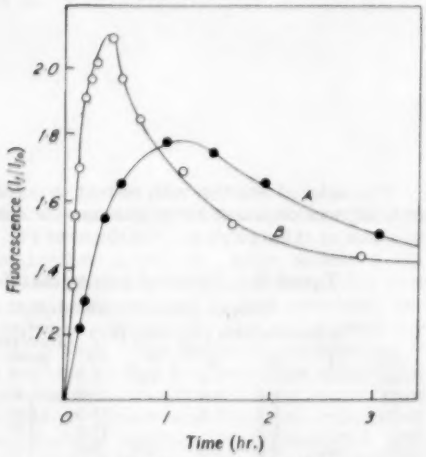
*C*, in 10% methanol–ether, with $10^{-2}$ mole/l. of $LaCl_3$.

FIG. 7. *Decrease in fluorescence on allomerization of chlorophyll-a* ($5 \times 10^{-6}$ *mole/l.*).




*A*, with $10^{-6}$ mole/l. of $LaCl_3$.
*B*, with $5 \times 10^{-6}$ mole/l. of $LaCl_3$.

FIG. 8. *Increase in fluorescence on allomerization of chlorophyll-b* ($5 \times 10^{-6}$ *mole/l.*).




*A*, with $10^{-6}$ mole/l. of $LaCl_3$.
*B*, with $10^{-4}$ mole/l. of $LaCl_3$.

chloride (Fig. 7) and other salts. Correction for the fractions of 0·553 and 0·427 of the incident light absorbed by chlorophyll-*a* and its allomer, respectively, gives a relative fluorescence intensity of the allomer of 71%. Three further corrections are required for a determination of relative yield owing to : (i) re-absorption of the measured fluorescence of the allomer, (ii) change of wavelengths affecting photocell sensitivity, and (iii) different fractions of the fluorescence spectrum being cut off by the photocell filter. The red absorption maximum moved 100 Å to shorter wavelength. An approximately mirror-image fluorescence spectrum with a similar shift being assumed, factors (i) and (ii), but not (iii), are likely to be unimportant; concerning (iii), about 30% less area under the fluorescence spectrum curve is estimated to be transmitted

by the filter. These measurements therefore indicate that allomerized chlorophyll-*a* possesses a fluorescence of an intensity of the order of that of chlorophyll-*a*.

In contrast with chlorophyll-*a*, the measured fluorescence intensity increased on the allomerization of chlorophyll-*b*. In absence of added catalyst or with accelerated allomerization, a maximum in fluorescence was obtained (Fig. 8), after which more extensive decomposition was indicated by absorption spectra. On correction for the fractions of 0·227 and 0·338 of the incident light absorbed by $5 \times 10^{-6}$ M-chlorophyll-*b* and its allomer, respectively, the maximum of fluorescence was 158% of the initial value. Arguments on probable wavelength changes of

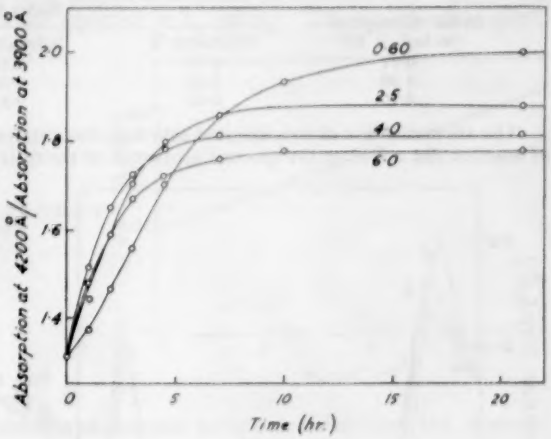



FIG. 9. *Allomerization of different concentrations of chlorophyll-a.*

(The numbers on the curves refer to initial chlorophyll concentrations $\times 10^3$ mole/l.)

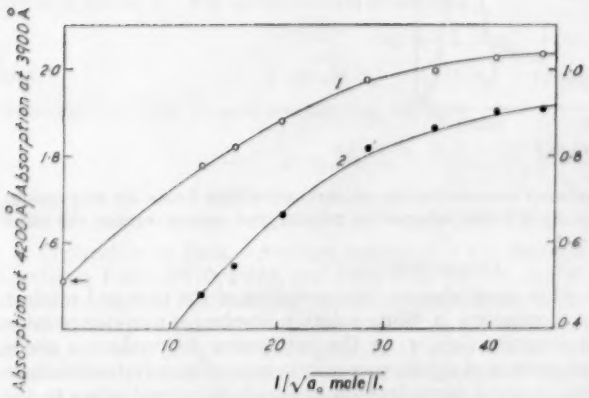



FIG. 10.

*Curve* 1. *Ratios of absorption at* 4200 Å/3900 Å *on allomerization, s, for different initial chlorophyll-a concentrations.*

*Curve* 2. *Calculated molar fractions of substance* X.

The arrow refers to the point $(s_{X'} + s_{X''})/2$

fluorescence indicate that this figure would be increased on making the further corrections necessary for relative yields.

*Concentrated Chlorophyll-a Solutions.*—With concentrations of chlorophyll-*a* from $5 \times 10^{-4}$ to $5 \times 10^{-5}$ M, the spectra on complete allomerization were identical and the rates were dependent on the chlorophyll concentration to the first power. However, at higher concentrations different behaviour was noted: (i) the initial rate of reaction as measured by the spectral method increased markedly with chlorophyll concentration (Fig. 9) and (ii) the spectral changes became static at 4200 Å/3900 Å ratios well below that for complete allomerization in more dilute solution (Figs. 9 and 10, curve 1).

In accord with these indications of more complex reaction, concentrated chlorophyll solutions after allomerization yielded two major products on chromatography in addition to the main product obtained with dilute solutions; the bands for unaltered chlorophyll and for these three products clearly separated on elution. Their spectra are recorded in Fig. 11.

Selective elution was attempted, although with too short columns to be more than semi-quantitative (the main objective being a first chromatography for spectra determination). After application of the approximate equality of extinction coefficients at 3900 Å, the proportions of the other two products compared with that from dilute solutions increased with initial chlorophyll concentration (Table 3). This is empirically the explanation of the decrease in final 4200/3900 Å ratio with chlorophyll concentration.

TABLE 3. *Approximate molar fractions of products after allomerization of chlorophyll-a solutions of different concentrations in methanol.*

| Initial chlorophyll-*a* (mole/l. × $10^3$) | Molar fractions of | | |
|---|---|---|---|
| | Substance X | Substance Y′ | Substance Y″ |
| 0·67 | 0·77 | 0·13 | 0·10 |
| 3·96 | 0·43 | 0·34 | 0·22 |
| 6·70 | 0·31 | 0·35 | 0·34 |

The allomerization of concentrated solutions was catalysed by salts, but the increase in rate of reaction did not alter the spectral properties of the final product. For example, a 4 × $10^{-3}$ M-solution of chlorophyll-*a* in methanol was completely allomerized within 5 min. by magnesium methoxide to give a spectral ratio, $s_f$, of 1·846, whereas an uncatalysed control reached the same ratio after 30 hr.

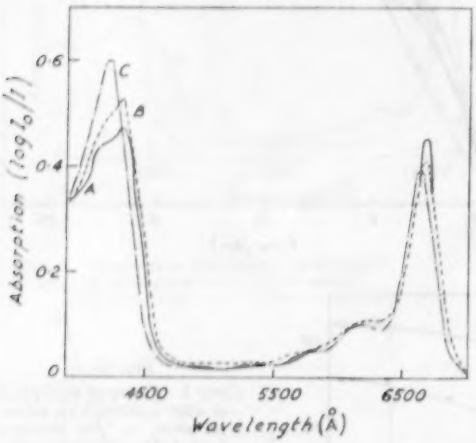



FIG. 11. *Absorption spectra of (A) chlorophyll-a, (B) substance Y′, and (C) substance Y″ in methanol.*

## DISCUSSION

*Uncatalyzed Allomerization.*—The most obvious interpretation of the rate and product results is that the predominant mechanism in dilute solution involves a rate-determining step dependent on chlorophyll concentration, $a$, to the *first* power to produce a single product, X, and that a second mechanism of significance only in more concentrated solutions involves a rate-determining step or steps dependent on chlorophyll concentration to the *second* power to produce the two further products, Y′ and Y″:

$$A \longrightarrow X \qquad -da/dt = k_1 a \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

$$2A \longrightarrow Y' + Y'' \equiv Y \qquad -da/dt = k_2 a^2 \quad . \quad . \quad . \quad . \quad . \quad (4)$$

The following analysis shows that the product and rate data are consistent with the schematic mechanism of (3) and (4). The composite product symbol Y is introduced merely to condense later algebraic equations; Y is of concentration equivalent to the sum of Y′ and Y″, and of a 4200/3900 Å spectral ratio, $s_Y$ equal to the arithmetic mean of the ratios for Y′ and Y″.

First to be considered are the concentrations, $x$ and $y$, of substances X and Y expected at the end of reaction for various initial chlorophyll concentrations, $a_0$, according to equations (1) and (2).

The final spectral ratio, $s_f$, is related to $x$, $s_X$, and $s_Y$ by :

$$x/a_0 = (s_f - s_Y)/(s_X - s_Y) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

Also, $$(dx/dt)/(-da/dt) = k_1a/(k_1a + k_2a^2)$$

from which, on complete reaction,

$$x = 2{\cdot}303(k_1/k_2) \log_{10} (1 + k_2a_0/k_1) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

Equation (6) provides values of $k_1/k_2$ from $x$ determined by equation (5). Initial rate data are next used to provide a second equation in $k_1$ and $k_2$. This requires chlorophyll

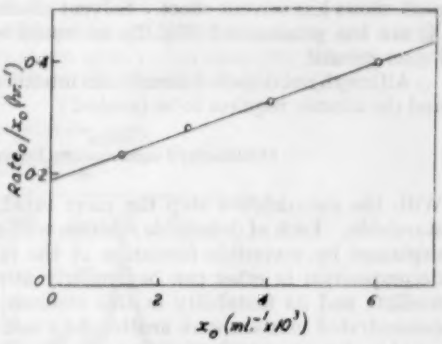



FIG. 12. *Initial rates of allomerization for different initial chlorophyll-a concentrations.*

concentrations at measured early periods of reaction to be calculated from the observed spectral ratios, $s$. For small extents of reaction,

$$x/y = k_1/k_2a_0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

and $$s = s_A(a/a_0) + s_X(x/a_0) + s_Y(y/a_0) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (8)$$

Substituting (7) in (8) and rearranging, we have

$$a = \frac{a_0[s - s_X + (s - s_Y)\, k_2a_0/k_1]}{[s_A - s_X + (s_A - s_Y)\, k_2a_0/k_1]} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (9)$$

Finally, $$(-da/dt)_0/a_0 = k_1 + k_2a_0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (10)$$

*Application to Data.*—Average values of $s$ for chlorophyll-*a*, X, Y′, and Y″ were respectively 1·334, 2·070, 1·418, and 1·597 from 18, 7, 9, and 6 samples separately isolated by chromatography. Introducing theses in equation (5), with the values of $s_f$ from Fig. 10, curve 1, we have the yields of $x$ for various $a_0$ recorded in Fig. 10, Curve 2. The curve is of eqn. (6) with $k_1/k_2 = 0{\cdot}0024$ mole/l. Values of $s$ at different times from Figs. 9 and similar data provide the initial rates of conversion of Fig. 12. In accord with eqn. (10), linearity of $-(da/dt)_0/a_0$ versus $a_0$ is obtained, to give values of $k_1$ and $k_2$ at 35° of $5{\cdot}1 \times 10^{-5}$ sec.$^{-1}$ and $1{\cdot}0 \times 10^{-2}$ sec.$^{-1}$ mole$^{-1}$ l., respectively.

Yields of X and Y on complete allomerization are given by eqn. (6), *i.e.*, curve 2 of Fig. 10. The experimental value of $k_1/k_2 = 0{\cdot}0024$ mole/l. gives 90% and 50% yields of X for initial chlorophyll concentrations of 0·57 and $5{\cdot}3 \times 10^{-3}$ mole/l., respectively. The approximate yields of X, Y′ and Y″ on chromatographic separation (Table 3) are of the order calculated from the initial chlorophyll concentrations.

*Catalyzed Allomerization.*—Variation of cation and anion among series of salts clearly shows that the former is the catalyzing species. The order of reaction with respect to salt concentration for the one salt investigated, 0·65 for magnesium chloride, appears somewhat unrevealing but is consistent with catalytic activity of the free cation of a partially dissociated salt.

This leads to some general considerations of chlorophyll reactivity and structure

influenced by small concentrations of cation. In contrast with the homopolar chemistry usually envisaged for chlorophyll, catalyzed allomerization is more similar to ionic or ion–dipolar molecule reactions. Two formal structures for a dipolar chlorophyll mloecule are suggested by the classical formula, a positive central magnesium and an anionic porphyrin ring, and an enol form of oxygen of ring v.[8] The cation may function as catalyst by formation of the metal enolate.

The spectra of chlorophyll-*a* in different solvents also indicate a polar tautomeric species (see Fig. 2). Methanol solutions show almost equal heights of the two peaks of the blue band and of the red peak, for chlorophyll-*a*. Ascent of the alcohol series greatly reduces the relative height of the minor blue peak. The relative absorption of the red peak shows less solvent effect. Solvent effects on the spectrum of the allomerized product, X, are less pronounced (Fig. 2), as would be expected if a tautomeric species were no longer present.

Although not detected directly, an intermediate between the reactive form of chlorophyll and the allomer requires to be invoked :

$$t\text{-Chlorophyll} \underset{}{\overset{\text{Cation}}{\rightleftharpoons}} \text{Intermediate} \xrightarrow[\text{Solvent}]{O_2} \text{Allomer}$$

With the autoxidative step the more rapid, insensitivity to oxygen pressure is understandable. Lack of detectable reaction with cation in absence of oxygen is most plausibly explained by reversible formation of the intermediate as the catalysed reaction. The decomposition in ether can be similarly attributed to catalyzed production of an intermediate and its instability in this medium. Finally, since the products of reaction in concentrated solution were unaltered by salt catalysis, a chlorophyll molecule itself seems capable of reacting with the intermediate. Thus the apparently independent allomerization processes in dilute and concentrated solution may be related.

The encouragement of Dr. R. S. Livingston, of the University of Minnesota, Minneapolis, U.S.A., and Dr. W. Gerrard of the Northern Polytechnic, London, is gratefully acknowledged. Facilities for research were also provided at the National College of Food Technology, London.

The Northern Polytechnic, Holloway Road, London, N.7. [*Received, October 26th*, 1955.]

[8] Rabinowitch, "Photosynthesis," Interscience Publ., New York, 1945, Vol. I, p. 459.

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## 252. *Cellular Constituents. The Chemistry of Xanthine Oxidase. Part II.* The Homogeneity of Crystalline Metalloflavoprotein Fractions.*

By P. G. Avis, F. Bergel, R. C. Bray, D. W. F. James, and K. V. Shooter.

The problem of homogeneity of crystalline molybdenoflavoprotein fractions [1] with high xanthine oxidase activity has been studied by sedimentation experiments, by electrophoresis, and by "constant protein" and "constant solvent" solubility analysis. All these tests, although in some instances not producing complete information, indicated that in our best preparations at least 90% of the material acted physicochemically as a single component. However, enzymic activity measurements, more fully discussed in Part III (following paper), made it clear that "active" and closely related "inactive" flavoproteins were present in this "component." From the sedimentation, diffusion, and density data, it has been calculated that the crystalline material has *M* about 290,000. Its isoelectric point is at pH 5·3—5·4.

Avis *et al.*[1] recently described a procedure for the preparation from cow's milk of a molybdenoflavoprotein with high xanthine oxidase activity and a low protein–flavin ratio, obtained for the first time in a crystalline form. It appears that xanthine oxidase, like

* Part I, *J.*, 1955, 1100.

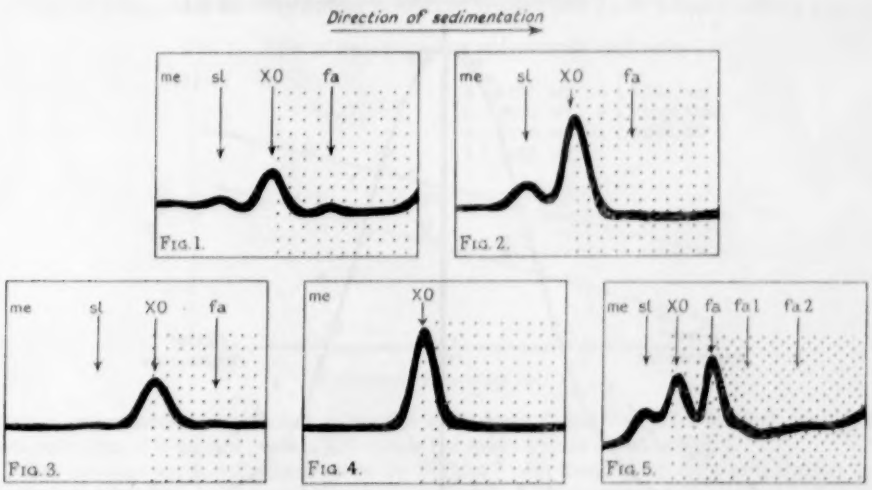
[1] Avis, Bergel, and Bray, *J.*, 1955, 1100.

other enzymic proteins, especially those with coenzyme- and metallo-prosthetic groups, decreases in activity during purification and on prolonged storage or mild chemical treatment, often without any obvious alteration of physicochemical properties. This may be due either to partial loss of its prosthetic groups, particularly of molybdenum, or to slight changes in its protein moiety.[2]

We now report below results of tests for homogeneity of some of our crystalline products in comparison with less pure fractions of our own and of other authors. The symbols M1—10 refer to numbers of different stages of the preparative procedure (see Avis *et al.*[1]), and LXO51—68 to those of the total batches, each from 20—40 l. of buttermilk. The xanthine oxidase activities of the various samples, expressed as " AFR " and " APR ", are given in Table 2, with other details of the specimens used.

*Sedimentation.*—Solutions of our molybdenoflavoprotein (LXO56, this being the batch described in Part I [1]), obtained at four successive stages of its preparation (M5, M6, M7, M8), have been examined in the ultracentrifuge. The sedimentation diagrams (Figs. 1—4)

FIGS. 1—5. *Sedimentation diagrams.*




clearly show the increase in the proportion of xanthine oxidase (peak " XO ") that was achieved at each step of the purification procedure. In the early stages, M5 and M6 (Figs. 1 and 2 respectively), considerable amounts of two major impurities (labelled " *sl* " and " *fa* ") were present, together with some polydisperse material of low molecular weight which produced an ill-defined peak between the meniscus (" *me* ") and the " *sl* "-peak. In the crystalline flavoprotein M7 (Fig. 3), the material of low molecular weight was absent and the greater portion of the " *sl* "- and the " *fa* "-component had disappeared. After recrystallisation leading to M8, the " *sl* "- and the " *fa* "-component were not detectable (Fig. 4).

From the changes in photographic density along the length of the sedimentation diagrams (indicated in the Figures by dotted areas), it could be deduced that the red-brown colour of the solution was associated, as would be expected, with the " XO "-peak. The " *sl* "-component was not coloured, but absorbed strongly in the ultraviolet region (250—265 mμ). Component " *fa* ", as shown in the diagram (Fig. 5) of one of the rejected fractions, S4 (see Avis *et al.*[1]), which contained an increased proportion of this substance,

[2] Cf. Corran *et al.*, *Biochem. J.*, 1938, **32**, 2231; 1939, **33**, 1694; Morell, *ibid.*, 1952, **51**, 657; Mahler and Green, *Science*, 1954, **120**, 7; Richert and Westerfeld, *J. Biol. Chem.*, 1954, **209**, 179; Mackler, Mahler, and Green, *ibid.*, 1954, **210**, 149; de Renzo, *Arch. Biochem. Biophys.*, 1954, **49**, 242; Mahler, Fairhurst, and Mackler, *J. Amer. Chem. Soc.*, 1955, **77**, 1514.

apparently possessed a colour similar to that of "XO". The two additional peaks in this diagram, "*fa* 1" and "*fa* 2", probably represent aggregates, resulting from incomplete dissolution. These results suggest that both "XO" and the impurity, "*fa*", contributed to the absorption of less purified fractions at 450 mμ ("flavin", see Avis *et al.*[1]).

The sedimentation coefficients ($s_{20,w}$) calculated for the three main components (see Experimental section) are given in Table 1.

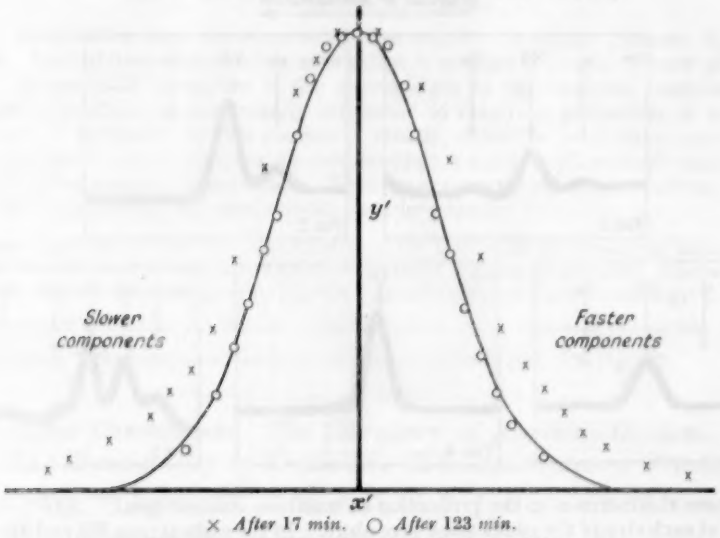
TABLE 1.

| Stage | M5 | M6 | S4 | M7 | M8 |
|---|---|---|---|---|---|
| $10^{13}s_{20,w}$: "*sl*" | 6·9 | 6·2 | 6·95 | * | † |
| "XO" | 11·5 | 11·0 | 11·5 | 11·3 | 10·8 |
| "*fa*" | 15·7 | * | 16·5 | * | † |

* Amount too small for measurement of sedimentant constants. † Not detected.

From Figs. 3 and 4, it can be deduced that 95% or more of our crystallisates in solution sedimented to form a single peak, "XO". However, on closer inspection of the sediment-

FIG. 6. *Normalised sedimentation diagram* (42,000 r.p.m.).




× *After* 17 *min.* ○ *After* 123 *min.*
*The curve is calculated from the diffusion coefficient.*

ation results, it was found that the corrected areas under the single peaks diminished as sedimentation proceeded, indicating the presence of polydisperse "impurities".[3] For stage M7, the decrease in area after 55 minutes' centrifugation amounted to 15%, only a third of which could be accounted for by the small quantities of "*sl*" and "*fa*" still present in this material. This preparation contained, therefore, in addition, at least 10% of polydisperse "impurities". Recrystallisation, leading to M8, reduced the total "impurities", since centrifugation for the same time as for M7 produced only a 5% decrease in area. After more prolonged centrifuged (2 hr.) it was calculated that M8 contained 10% of total impurities.

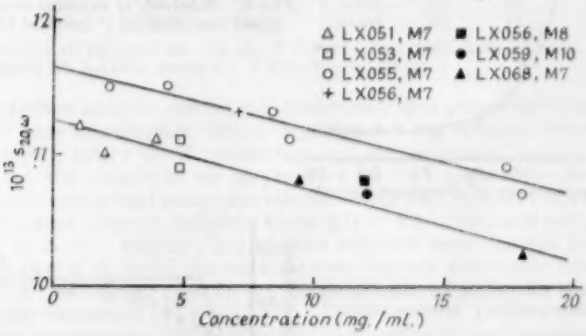
The presence of these small amounts of extraneous material can be discerned still more clearly when "normalised" sedimentation curves are considered.[4] When this method is applied to fraction M8, Fig. 6 discloses that at the beginning of the sedimentation process the spread of the peak was greater than could be accounted for by calculation from

[3] Svedberg, Pedersen, Lamm, and Kraemer, "The Ultracentrifuge," Oxford Univ. Press, 1940.
[4] Davison, James, Shooter, and Butler, *Biochim. Biophys. Acta*, 1954, **15**, 416.

our diffusion values for the " XO "-component (see p. 1218). But after 2 hours' sedimentation the theoretical curve and that calculated from experiment agreed well, which meant that the polydisperse " impurities " had disappeared from under the main peak. This confirmed our estimate of the amount of impurity in M8 arrived at by the area method given above. It also showed that some of the " impurities " sedimented faster and some slower than the main component.

The sedimentation coefficients for the " XO " peak have been measured on seven crystalline preparations from different batches at varying concentrations of the solution (Fig. 7; M7 of LXO51, 53, 55, 56, 68, M8 of LXO56, and M10 of LXO59). Extrapolation of these results to zero concentration gives an average $s_{20,w} = 11{\cdot}4 \pm 0{\cdot}3 \times 10^{-13}$ sec. It appears that the sedimentation coefficients of different batches may vary slightly, but significantly (two separate lines as seen in Fig. 7), perhaps as the result of the presence of different amounts of polydisperse " impurities " or because of their varying xanthine oxidase activities (cf. following paper, also Table 2 and the remark on the loss of activity through storage, p. 1217). It may be noted that one of our specimens, derived from washings of LXO60, M7, which had almost completely lost its xanthine oxidase but not its DPNH-dehydrogenase activity during prolonged storage (see Table 2 and following paper),

FIG. 7. *Effect of concentration on sedimentation coefficients.*




gave a sedimentation coefficient $s$ for the main peak " XO " of $11{\cdot}2 \times 10^{-13}$ sec. at a concentration of 3 mg./ml., which falls inside the lower values noted in Fig. 7.

Our findings agree with those given by Philpot [5] who based them on a preparation by Corran *et al.*[2] (1939). He found three components, which correspond to our " *sl* ", " *fa* ", and " XO ".

*Molecular Weight.*—The molecular weight has been calculated by using the equation $M = \boldsymbol{R}Ts/(1 - \bar{v}\rho)D$ (Svedberg *et al.*[3]). Combining the extrapolated value for $s_{20,w}$ (see above) with the diffusion constant, $D = (3{\cdot}6 \pm 0{\cdot}2) \times 10^{-7}$ cm.$^2$ sec.$^{-1}$, and the partial specific volume, $\bar{v} = 0{\cdot}74 \pm 0{\cdot}02$ c.c./g. (see p. 1218), one obtains $M = 290{,}000$, and after allowance for the extreme limits of error in $s$, $D$, and $\bar{v}$ the maximum possible error is $\pm 40{,}000$. This value of $M$ agrees with that deduced by Philpot [5a] from a measured value of $s$ and assumed values for $D$ and $\bar{v}$, *viz.*, $M = 220{,}000$—$320{,}000$. The molecular weight, obtained from chemical analysis of the enzyme and agreeing with the above, is discussed in the following paper.

The axial ratio of the molecule calculated from the diffusion coefficient [3] is 7 : 1 (on the assumption that there is no hydration).

*Electrophoresis.*—The only electrophoretic work on purified milk xanthine oxidase reported to date is that of Ball [6] who used Theorell's technique; [7] he found that the isoelectric point of his enzyme preparation was at pH about 6·2. No details of experimental conditions were given.

[5] Philpot, *Biochem. J.*, (*a*) 1939, **33**, 1707; (*b*) 1938, **32**, 2240.
[6] Ball, *J. Biol. Chem.*, 1939, **128**, 51.
[7] Theorell, *Biochem. Z.*, 1934, **275**, 1.

We have examined specimens of our crystalline fractions in an apparatus of the Tiselius type. For LXO60, M7 in preliminary experiments no migration was observed after 6 hr. at pH 5·3. When LXO62, M7 was examined at pH 4·50, 5·28, 5·46, 6·32, and 8·15 some precipitation was noted at the lowest pH, and at the highest, gelation occurred. From the mobilities it was concluded that the isoelectric point was between pH 5·28 and 5·46 (acetate buffer, $I = 0{\cdot}2$). Since in some of these experiments, particularly in that at pH 6·32, the single peak observed was slightly skew, indicating the presence of some impurities, a further test was done with a purer specimen LXO59, M10 which migrated as a single symmetrical peak after 5 hr. at pH 6·6. As the apparatus available to us did not warrant a more detailed analysis of the boundary curves (cf. Alberty [8]), these electrophoretic results were not adequate to elucidate the finer aspects of homogeneity of our preparations.

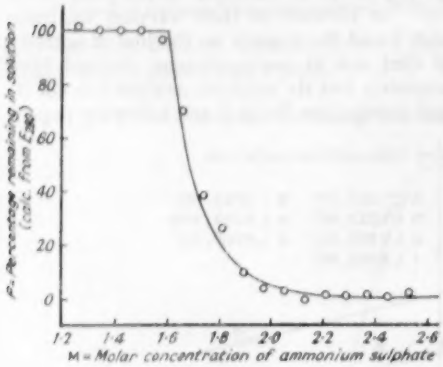
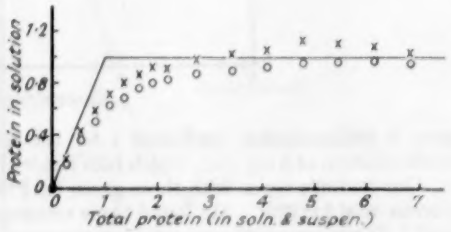



FIG. 8. *Solubility of xanthine oxidase as a function of salt concentration ("constant protein" method).*

FIG. 9. *Effect of total protein concentration on the solubility of xanthine oxidase ("constant solvent" method).*

*Protein concns. in arbitrary units:*

○ *from* $E_{280}$;
× *from enzyme assays.*




*Solubility Analyses.*—Two tests for homogeneity of proteins are available, the "constant solvent" test [9] and the "constant protein" test.[10] In the "constant protein" method sample LXO59, M10 gave an exponential curve (Fig. 8) showing no breaks, in agreement with the theory for a single protein component. However, for this sample in the "constant solvent" solubility test some deviation from the ideal behaviour was found (Fig. 9) when the dissolved protein was estimated by means of xanthine oxidase activity or ultraviolet absorption at 280 mμ. Since fairly rapid loss of activity occurred under our conditions, which allowed the measurements to be carried out only at one time of equilibration between solids and solutions, no certainty existed that final equilibrium had been reached and that the results were therefore significant.

*Conclusions.*—The physicochemical data obtained from sedimentation experiments and from "constant protein" solubility analysis show that, at this stage of our work on crystalline molybdenoflavoprotein fractions from milk, we are dealing with preparations in which at least 90% of the material is behaving as a single component. While the observation of single peaks in the electrophoretic tests at several pH values supports this

[8] Alberty in "The Proteins," ed. Neurath and Bailey, Acad. Press, New York, 1953, Vol. IA, p. 461.
[9] See Herriot, *Chem. Rev.*, 1942, **30**, 413.
[10] See Cohn, *Physiol. Rev.*, 1925, **5**, 349.

conclusion, one is forced by the variation in xanthine oxidase activity to assume the presence in the " component " of at least two closely related molecular species.

## Experimental

*Flavoprotein Solutions.*—The fractions used for the physicochemical measurements were derived from batches, prepared according to the procedures described by Avis *et al.*,[1] the batches being designated LXO51 to LXO68. The different stages of the purification were given the symbols M1—M10 and S1—S7 as explained for most of them in the previous communication,[1] where in fact LXO56 was the batch in question. No mention was made previously of M9 and M10; the former was obtained by recrystallisation of M8, and the latter by the same process from M9, as reported in detail for M8 by Avis *et al.*[1] The ratios " AFR ", " APR ", and " PFR " for the different batches are given in Table 2, and refer to the assay with xanthine as substrate

Table 2. *Properties of various batches.*

| Batch | Stage | " AFR " [a] | " APR " [a] | " PFR " [a] | Batch | Stage | " AFR " [a] | " APR " [a] | " PFR " [a] |
|---|---|---|---|---|---|---|---|---|---|
| LXO51 | M6 [b] | 62 | 7·3 | 8·5 | LXO56 | M8 [c] | 71 | 14·2 | 5·0 |
| LXO51 | M7 [b] | 53 | 10·4 | 5·1 | LXO59 | M5 | 79 | 7·9 | 9·9 |
| LXO53 | M7 [b] | 32 | 6·1 | 5·3 | LXO59 | M10 | 43 | 8·8 | 5·0 |
| LXO55 | M7 | 69 | 12·4 | 5·6 | LXO60 | M7 | 70 | 14·0 | 5·0 |
| LXO56 | M5 [c] | 70 | 7·4 | 9·4 | LXO60 | *ex* M7 | 0·3 | 0·04 | 6·8 |
| LXO56 | M6 [c] | 88 | 11·1 | 8·0 | LXO62 | M7 | 28 | 5·2 | 5·4 |
| LXO56 | M7 [c] | 79 | 15·2 | 5·2 | LXO68 | M7 | 18 | 3·1 | 5·7 |

[a] For definition of symbols see ref. 1. [b] Values given previously (*Nature*, 1954, **173**, 1230) • LXO51 was batch B; LXO53, batch A. [c] Values given in ref. 1.

and oxygen as electron acceptor, carried out immediately after preparing the solution. In some instances there were considerable delays, accompanied by variable reductions of activity (cf. following paper), before the ultracentrifuge and electrophoresis experiments were carried out. In all cases, the samples of the preparations for the experiments described in this and the following paper were stored as aqueous solutions with total protein concentrations of about 1—2%, in the presence either of phosphate buffer (pH 6; 0·1—1·0M) or of sodium chloride (0·2M; pH near to 6), at 0—5°. Aliquot parts of these solutions were removed for use as required. For measurements of $s$, $D$, and $\bar{v}$, the solutions were dialysed thoroughly against 0·2M-sodium chloride at 0—5° before use. Before the electrophoretic experiments dialysis against the appropriate buffer was carried out.

*Sedimentation Experiments.*—These were performed with a Spinco analytical ultracentrifuge, running at speeds of 42,000 or 50,000 r.p.m. Photographs of the boundary formed between solution and solvent during sedimentation were taken with the Philpot–Svensson optical system.[11] Visible light was used, except in one experiment with ultraviolet light. The concentrations of the xanthine oxidase solutions were determined from the absorption at 450 mμ, the absorption coefficient $E^{1\%}_{1\,cm.}$ (450 mμ) being 2·3 (cf. following paper). For a few solutions, absorption data were not available, and then the concentrations were estimated by comparing the areas under the peaks of the sedimentation diagrams with the corresponding area for a solution of known concentration. These areas were obtained from enlargements of the boundary photograph drawn on graph paper, and were corrected for the dilution effect resulting from the use of a sector-shaped cell.[3]

The sedimentation coefficients were calculated from the rate of movement of the maximum of the peak in the usual manner and were then corrected for the adiabatic cooling of the rotor during acceleration, the variation of the viscosity of the solvent with temperature, and the difference between the viscosity of the solvent and of water, to give the sedimentation coefficient $s_{20,w}$ in water at 20° (see Table 1 and Fig. 7). Previous experiments have shown that $s$ can in general be determined with an accuracy of ±2% with this apparatus.

Two methods of determining the degree of purity of the crystalline preparations were used: (*a*) The area under the peak of the sedimentation diagram, after different times of sedimentation, was measured as described above. (*b*) [4] The peaks were drawn on graph paper (see above); the $y$ axis was taken as a line drawn vertically through the maximum of the peak, and the $x$ axis as the base of the peak. The peaks were then normalised by means of the transformations $x' = xt^{-\frac{1}{2}}$ and $y' = yt^{\frac{1}{2}} \exp 2w^2st$. With LXO56, M8 (Fig. 6) the theoretical curve was calculated from $D = 3{\cdot}5 \times 10^{-7}$ cm.$^2$ sec.$^{-1}$.

[11] Philpot, *Nature*, 1938, **141**, 283; Svensson, *Kolloid Z.*, 1939, **87**, 181.

*Diffusion.*—For three of the experiments (at 20°), with LXO68, M7, the Hilger electrophoresis apparatus fitted with a tungsten lamp and a red filter (Wratten 29) was used. Photographs were taken at intervals up to 3—5 days on Kodak IRER plates with the Philpot–Svensson optical system. The boundaries, between the solvent and the enzyme solution, formed by sliding the two halves of the electrophoresis cell together, were slowly moved into the centre of the cell and carefully sharpened. One experiment with LXO56, M7 (at the same temperature) was performed with the Perkin-Elmer electrophoresis apparatus and the Longsworth scanning technique. The diffusion coefficients were calculated by the height–area method.[13]

For LXO68, M7 it was found that at concentrations of 1·75, 0·94, and 0·55% of the protein, $10^7\ D_{20,w} = 3{\cdot}74 \pm 0{\cdot}12$, $3{\cdot}57 \pm 0{\cdot}07$, and $3{\cdot}51 \pm 0{\cdot}14$ cm.$^2$ sec.$^{-1}$ respectively. For a 0·5% solution of LXO56, M7, it was 3·5 cm.$^2$ sec.$^{-1}$. Since the diffusion coefficient did not vary with concentration, within the experimental error, the mean value of $D_{20,w} = (3{\cdot}6 \pm 0{\cdot}2) \times 10^{-7}$ cm.$^2$ sec.$^{-1}$ was used in the calculations of molecular weight.

*Partial Specific Volume.*—The densities of a sample of LXO68, M7 and of the 0·2M-sodium chloride against which it had been dialysed, were measured at 21°, with a 3 ml. pycnometer.[13] The concentration of the solution (2·05%) was determined from the value of $E_{280}$ (following paper). The density of the solution was found to be 1·0111 and that of the solvent 1·0059. The value of the partial specific volume was therefore $\bar{v} = 0{\cdot}74 \pm 0{\cdot}02$ (the limits of error on this quantity allow for weighing errors and for possible errors in the $E^{1\%}_{1\text{cm.}}$ value given in the following paper). It is assumed [13] that $\bar{v}$ equals $\phi$.

*Electrophoresis.*—Buffer solutions were made up to a constant ionic strength ($I$ 0·2) according to the data given by Miller and Golder.[14] Solutions of xanthine oxidase at a concentration of about 0·8% were employed.

Electrophoresis was performed with a Perkin-Elmer apparatus fitted with the Longsworth optical measuring system,[15] working at 0° and a constant current of 15 milliamp. Since the solutions were deep red the photographs of the boundary were taken on the same type of red-sensitive photographic plates, as for the diffusion experiments (above). Conductivities were measured at 0° with a Leeds–Northrup bridge. LXO62, M7 migrated cathodically at pH 4·5 (electrophoretic mobility $\mu = 1{\cdot}7 \times 10^{-5}$ cm.$^2$ v$^{-1}$ sec.$^{-1}$) and with lower mobility in the same direction at pH 5·28. At pH 5·46 the migration was anodic ($\mu = 0{\cdot}09 \times 10^{-5}$ cm.$^2$ v$^{-1}$ sec.$^{-1}$), with increasing mobility at pH 6·32 ($\mu = 2{\cdot}9 \times 10^{-5}$ cm.$^2$ v$^{-1}$ sec.$^{-1}$).

*"Constant Protein" Solubility Test (Effect of Salt Concentration).*[10]—The sample LXO59, M10 was diluted with phosphate buffer of pH 6, and portions (2 ml.) of the diluted solution were placed in a series of centrifuge tubes, together with varying quantities (0—4 ml.) of 50% w/v aqueous ammonium sulphate, and 25% w/v ammonium sulphate to bring the total volume in all samples up to 6 ml. The tubes were closed with rubber caps, and mixed by inversion; after 20 hr. at about 3° with very occasional inversion to assist equilibration, the tubes were centrifuged (10 min. at 6000 *g* and +3°). 2 ml. of each of the clear supernatant liquids were removed and diluted with water (2 ml. portions), and the light absorption at 280 mμ measured in a 4 cm. cell with a plastic fitting designed to reduce the volume of liquid required. Small corrections for the low absorption due to ammonium sulphate were applied,[16] the corrections being calculated from measurements on the stock 50% and 25% ammonium sulphate solutions. The percentage of protein remaining in solution (Fig. 8) was calculated from these $E_{280}$ values, the $E$ values corresponding to 0 and 100% precipitation being determined empirically. The molarities of ammonium sulphate were calculated by ignoring volume changes on mixing of the solutions. In this experiment, the initial protein content was about 0·2 mg./ml., the final phosphate concentration was 0·3M, and the calculated pH in the final concentrated salt solutions was about 5. The curve through the experimental points in the Figure was calculated from Cohn's equation in the form: $M = A - B \log_{10} P$, where M is the molarity of ammonium sulphate, $P$ is the percentage of protein remaining in solution, and $A$ and $B$ are constants, taken here as 2·2 and 0·3, respectively.

*"Constant Solvent" Solubility Test (Effect of Protein Concentration).*—The sample LXO59, M10, after some weeks' storage, was dialysed against 0·1M-phosphate buffer (pH 6) and centrifuged to remove small amounts of precipitated material. Samples of the solution (up to 0·5 ml.) were placed in a series of centrifuge tubes, and portions of the buffer used in the dialysis

[13] Alexander and Johnson, "Colloid Science," Oxford Univ. Press, 1949.
[14] Cf. Koenig, *Arch. Biochem.*, 1950, **25**, 241.
[14] Miller and Golder, *ibid.*, 1950, **29**, 420.
[15] Longsworth, *Ind. Eng. Chem. Anal.*, 1946, **18**, 219.
[16] Derrien, *Biochim. Biophys. Acta*, 1952, **9**, 49.

were added to bring the total volume in all tubes to 0·5 ml. Portions (5 ml.) of a solution containing ammonium sulphate and phosphate buffer were added to each tube, which was then closed and equilibrated as for the " constant protein " determinations. All operations, including making up of the mixtures, were carried out at about +3°. The samples were centrifuged, the supernatant liquids were diluted, and the absorption at 280 mμ was measured as before. Aliquot parts of the diluted solutions were also assayed for xanthine oxidase activity (cf. ref. 1); from these figures the " protein in solution " values (Fig. 9) were obtained. The absorption and activity figures for the total protein, in solution and in suspension, were obtained by removing samples of a few of the suspensions, before the centrifugation, and diluting and measuring these in the same way as for the supernatant liquors. Graphs were plotted of $E_{280}$, or of activity, against the volumes of the enzyme solution originally introduced into the tubes and, from these, the " total protein " values for all the tubes were deduced. In the case of the $E_{280}$ measurements, the intercept of the graph on the $E$ axis also served to evaluate the blank correction, which was due to absorption by the salt mixture and had to be subtracted from both the " total protein " and the " protein in solution " figures. The maximum solubility found in this experiment corresponded to about 0·2 mg. of protein per ml.; the buffer concentration and pH were as in the previous experiment; the ammonium sulphate concentration was 1·66M.

We thank Professor A. Haddow for his continued interest, and Mrs. K. Tussler and Messrs. N. A. Perkins and T. Stebbings for technical assistance.

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## 253. *Cellular Constituents. The Chemistry of Xanthine Oxidase. Part III.* Estimations of the Co-factors and the Catalytic Activities of Enzyme Fractions from Cow's Milk.*

By P. G. AVIS, F. BERGEL, and R. C. BRAY.

Analyses for three co-factors of milk xanthine oxidase have been carried out on crystalline and less purified fractions.[1] The purest preparations † contained 8 atoms of iron, 2 molecules of flavin-adenine dinucleotide (FAD), and 1·3—1·5 atoms of molybdenum per molecule of protein. When assayed for enzymic activities these fractions exerted catalytic actions similar to those reported by other workers, except for certain quantitative differences. The latter were found most pronounced when reduced coenzyme I (DPNH) was used as substrate or when cytochrome-*c* was the electron acceptor; particularly in the second case, a very low activity was recorded.

PREVIOUS papers of this series [1,2] have described the preparation, from cow's milk, of crystalline and other xanthine oxidase fractions, and their examination for homogeneity. The present paper covers analyses of these fractions for the three co-factors, flavin-adenine dinucleotide (FAD), molybdenum, and iron, and measurements of the catalytic activities of selected samples.

*The Co-factors.*—At the time of writing the following values for these prosthetic groups have been published or can be calculated from data given by the authors: for FAD,† Corran *et al.*,[3] 0·465%, Morell,[4] 0·435%; Richert and Westerfeld,[5] for FAD † 0·49%, for Mo 0·03%, and for Fe 0·14%. Green and Beinert,[6] Totter *et al.*,[7] and Mackler *et al.*[8] have

* Part II, preceding paper.
† Calc. for the anhydrous free acid (*M* 786).

[1] Avis, Bergel, and Bray, *J.*, 1955, 1100.
[2] Avis, Bergel, Bray, James, and Shooter, preceding paper.
[3] Corran, Dewan, Gordon, and Green, *Biochem. J.*, 1939, **33**, 1694.
[4] Morell, *ibid.*, 1952, **51**, 657.
[5] Richert and Westerfeld, *J. Biol. Chem.*, 1954, **209**, 179.
[6] Green and Reinert, *Biochem. Biophys. Acta*, 1953, **11**, 599.
[7] Totter, Burnett, Monroe, Whitney, and Comar, *Science*, 1953, **118**, 555.
[8] Mackler, Mahler, and Green, *J. Biol. Chem.*, 1954, **210**, 149.

reported the ratio FAD : Mo as being 2 : 1, and Richert and Westerfeld [5] including their iron values give 2 : 1 : 8.

Our own values are recorded on Table 1. Our determinations of FAD (col. 3*a*) were carried out fluorimetrically and confirmed by microbiological estimations. The values for our crystalline samples (Nos. VI—IX) are of the same order as those published, although some of the latter (Corran *et al.*;[3] Morell [4]) are liable to be high as these workers were unaware, at the time, of the presence of iron which could contribute to the absorption at 450 mμ of their deproteinized solutions (cf. Richert and Westerfeld [5]). Calculating *M* from the highest FAD content, one obtains 154,000 for one FAD bound to one protein molecule. Doubling this figure for two FAD molecules per unit of protein one arrives at *M* 308,000, in agreement with the value derived [2] from sedimentation and diffusion data, namely *ca.* 290,000.

The iron content of the crystalline samples (VI—IX) varied little and averages, for the four figures recorded (columns 3*b* and 6), 4·0 atoms of iron per molecule of FAD, in agreement with Richert and Westerfeld's data.[5] This is supported by the constancy of the ratio $E^{450}_{1\,cm.}$ : Fe(μg./ml.) (0·164 ± 0·007).

While analyses of all preparations described in the literature appear to give a ratio of one atom of molybdenum to two molecules of FAD, our results (columns 3*c* and 7) provide some support for the suggestion by Mahler and Green [9] that native xanthine oxidase might contain one atom of molybdenum per molecule of FAD. In fact, this ratio has been claimed recently by Kielly [10] to exist in her preparations from calf and rat livers. However our ratios are fractional, with an average of 0·70 for our crystalline samples and a probable error for the individual ratios not greater than ±10%. The significance of these fractional ratios in terms of the structure of the enzyme is still obscure.

Unlike Mackler *et al.*,[6] we were unable to remove any substantial amount of molybdenum by dialysis of one of our fractions (LXO59, M5) against ammonia, and the sample retained at the same time almost all its catalytic activity (sample II, column 5). In contrast, another of our fractions (LXO60, ex M7, sample V), which after storage was found to be virtually devoid of xanthine oxidase activity and sedimented [2] similarly to active samples, still gave normal analytical results for the co-factors.[9]

TABLE 1. *Composition of xanthine oxidase fractions.*

| | 1 | 2 | 3 | | | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|---|
| | | | Percentage composition | | | | Activity units [c] | | |
| No. | Batch | Stage | (*a*) FAD | (*b*) Fe [c] | (*c*) Mo [c] | "PFR" [d] | per μmole of FAD | Fe (atoms) FAD (mols.) | Mo (atoms) FAD (mols.) |
| I | LXO59 | M5 | 0·19 | 0·06 | 0·020 | 9·9 | 3·7 | 4·6 | 0·87 |
| II | LXO59 | (M5) [a] | Solutions too turbid for accurate | | | | 3·5 | 4·9 | 0·81 |
| III | LXO56 | S4 | absorption measurements at 280 mμ | | | | 3·0 | 6·1 | 0·64 |
| IV | LXO51 | M6 | 0·31 | 0·08 | 0·021 | 8·5 | 2·1 | 3·5 | 0·56 |
| V | LXO60 | Ex M7 [b] | 0·36 | 0·10 | 0·028 | 6·8 | 0·01 | 4·1 | 0·64 |
| VI | LXO55 | M7 | 0·44 | 0·12 | 0·040 | 5·6 | 2·6 | 3·9 | 0·75 |
| VII | LXO56 | M7 | 0·47 | 0·14 | 0·040 | 5·2 | 2·9 | 4·0 | 0·70 |
| VIII | LXO60 | M7 | 0·48 | 0·14 | 0·038 | 5·0 | 2·6 | 4·0 | 0·64 |
| IX | LXO59 | M10 | 0·51 | 0·15 | 0·043 | 5·0 | 1·6 | 4·1 | 0·69 |

[a] After dialysis against ammonia (see Experimental). [b] "Inactive sample" (see Experimental). [c] Metals estimated by colorimetric methods (see Experimental). [d] *I.e.*, $E_{280}/E_{450}$ (see ref. 1). [e] Activity of freshly prepared solutions in X/O assay (see Experimental).

That no other metals formed part of the flavoprotein molecule was borne out by emission spectroscopy of sample LXO55, M7. While iron and molybdenum were present in about the same concentrations as calculated from our colorimetric data, all other metals which could be detected were found at concentrations less than one tenth of that of molybdenum.

The absorption spectrum of our material (LXO60, M7) was very similar to that given

[9] [*Added, March 20th, 1956.*]—A *crystalline*, inactive sample, similar to the above, has since been obtained.

[9] Mahler and Green, *Science*, 1954, **120**, 7.
[10] Kielly, *Fed. Proc.*, 1955, **14**, 235.

by Mackler *et al.*,[8] though our lower " PFR " indicated higher purity (column 4). The inactive sample (LXO60, ex M7) did not differ substantially from the above.

*Enzymic Activities.*—The main catalytic properties of our samples, in comparison with those in the literature,[11] are given in Table 2.

TABLE 2. *Rates of oxidation of various substrates catalysed by xanthine oxidase.*

Symbols: (*a*) Substrates: X = xanthine, H = hypoxanthine, A = acetaldehyde, D = DPNH. (*b*) Electron acceptors: O = oxygen, Mb = methylene-blue, I = 2 : 6-dichlorophenol-indophenol, Cy = cytochrome-c.

| 1 | 2 | 3 | 4 | 5 | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Absolute rates for reaction | | | Rates as % of corresponding X/O rate | | | | | |
| | | | | *a* | *b* | *c* | *d* | *e* | *f* |
| Sample | X/O | H/Mb [a] | A/Mb [a] | X/O | X/I | A/I | D/I | D/O | H/Cy |
| Corran *et al.*[3] | — | 1·8 | 2·6 | — | — | — | — | — | — |
| Morell [4, b] | 0·52 | — | — | — | — | — | — | — | — |
| Mackler *et al.*[8, c] | (1·25) | — | — | (100) | (106); 49 | (24) | (54) | 6 | 30 |
| LXO56, M7 [d] | 1·82 | 1·3; 2·3 [e] | — | — | — | — | — | — | — |
| LXO60, M7 (freshly prepared) | 1·68 | 1·1; 1·9 [e] | 2·4 | 100 | 41 | 12 | — | 0·7 | — |
| " (after 3—4 weeks) | 0·83 | — | — | 100 | 43 | 13 | 1·3 | — | 0·1 [f] |
| LXO60, M5 | 0·93 | — | — | 100 | 43 | 18 | 1·5 | 0·7 | 0·1 [f] |
| LXO61, M4 | 0·09 | — | — | 100 | 42 | 20 | 1·9 | 0·9 | 0·1 [f] |
| LXO61, M1 (buttermilk) | 0·02 | — | — | 100 | 29 | 10 | 3·6 | 1·5 [g] | — |
| LXO63, whole milk | — | — | — | 100 | — | — | 6·4 | — | — |
| LXO60; " inactive " sample (ex M7) | 0·005 | — | — | 100 | — | 10 | 400 | 200 | — |

Absolute rates as μmoles of substrate oxidised per mg. of protein per min. For conditions, see Table 3 and Experimental section. Figures in parentheses are extrapolations by the method of Lineweaver and Burk (*J. Amer. Chem. Soc.*, 1934, **56**, 658).

[a] Conditions as given by Corran *et al.*[3] [b] pH 8·3; 19°. [c] pH 7·0; 22°. [d] See ref. 1. [e] Obtained with a reduced hypoxanthine concentration. [f] Identical figures were obtained whether $MoO_4$ was present or absent. [g] Value from Batch LXO58, M1.

While our figures for X/O (column 2; see Table for explanation of symbols), representing observed rates and measured by spectrophotometric methods, were higher than those obtained by other workers, the methylene-blue activities, obtained in Thunberg tubes, with hypoxanthine (H/Mb) or acetaldehyde (A/Mb) (columns 3 and 4 respectively) were of the same order of magnitude as reported by Corran *et al.*[3] Other enzymic activities, expressed in percentages of the corresponding X/O rate on the same sample, such as X/I and A/I (columns 5*b* and 5*c* respectively) showed fair constancy for all fractions examined. Discrepancies from published data have been encountered by us with the reactions involving DPNH-dehydrogenation and cytochrome-*c* reduction, D/I, D/O, and H/Cy (columns 5*d*, *e*, and *f*, respectively). Our freshly prepared crystalline material showed lower activities for the first two than those given by Mackler *et al.*[8] A finding which has not been reported previously was that D/I and D/O decreased rapidly during the early stages (from whole milk to M4) of our purification process. On the other hand, DPNH-activity in more purified samples was observed to be more stable than the activities recorded in the preceding columns. The extreme case was that of sample LXO60, ex M7, mentioned before, which was virtually devoid of X/O and A/I activities (columns 2 and 5*c*), while retaining most of its D/I and D/O potencies. This is reminiscent of the results by Corran *et al.*[12] who described a milk flavoprotein related to xanthine oxidase with DPNH but no X/O activity.

Our figures for H/Cy (column 5*f*) add one more contradiction to those existing in the publications dealing with this enzymic activity.[8, 10, 13] Our own very low values for the

[11] See review by Singer and Kearny, " The Proteins," ed. Neurath and Bailey, Academic Press, New York, 1954, Vol. IIA, p. 225.

[12] Corran *et al.*, (*a*) *Biochem. J.*, 1938, **32**, 2231; (*b*) also ref. 3. Cf. (*c*) Philpot, *ibid.*, 1938, **32**, 2240; 1939, **33**, 1707; (*d*) and ref. 8.

[13] (*a*) Horecker and Heppel, *J. Biol. Chem.*, 1949, **178**, 683; (*b*) Morell, *Biochem. J.*, 1952, **51**, 666; *Biochem. Biophys. Acta*, 1955, **16**, 258; Mahler, Fairhurst, and Mackler, *J. Amer. Chem. Soc.*, 1955, **77**, 1514.

activities did not increase under anærobic conditions and in absence of salts, nor did they alter whether molybdic oxide was present or not.

*Discussion and Conclusions.*—For the co-factors of our crystalline specimens, the average ratios protein : FAD : Mo : Fe were 1 : 2 : (1·3—1·5) : 8. While FAD and iron appeared to be relatively securely bound, whatever the history of the sample, molybdenum was found in rather variable amounts. The effects of this variability on enzymic activities were not of a simple nature. Mahler and Green [9] postulated that molybdenum is not required for X/O activity, but only for transfer of electrons from specific substrates to single-electron acceptors, such as cytochrome-*c* or ferricyanide. This must be contrasted with the observation by de Renzo *et al.*[14] and by Richert and Westerfeld [5] that loss of molybdenum parallels loss of X/O activity. As to the latter, our results with samples, prior to crystallisation (showing some correlation between molybdenum content and this activity), and with crystalline fractions (showing no such correlation), left us undecided. But there is no doubt that loss of X/O and also X/I and A/I, but not D/O and D/I, activity cannot be caused solely by deficiency of molybdenum. Other modes of damage to the native molecule which still await elucidation must be operative, particularly in the protein moiety. The existence of a milk flavoprotein, having no X/O activity and related in chemical and physicochemical properties to active xanthine oxidase, points in this direction.[2, 12a, 12c, 15] Morell [4] (see his Fig. 2) has determined the amount of X/O inactive molecules in his enzyme preparations by two methods; our own observations [1, 2] on the variability of " AFR " values and of X/O activity per μmole of FAD (see Table 1, column 5) were in agreement with his results. So far, we have not applied his " immediate reduction " method.

Summarising, it can be assumed that our own preparations and those by other workers (which all contained larger amounts of extraneous proteins) still consist of mixtures of two or more of (1*a*) the fully active enzyme, (1*b*) an " inactive " enzyme with a modified protein moiety, possibly capable of catalysing DPNH-dehydrogenation, (2*a*) molybdenum-poor flavoprotein molecules, derived from the native enzyme, and (2*b*) molybdenum-poor molecules with a modified protein moiety; both (1*a*) and (1*b*) have a sufficient molybdenum content; both (2*a*) and (2*b*) are of unknown catalytic potency. While some of our xanthine oxidase preparations (*e.g.*, LXO56, M7) and that described by Corran *et al.*[3] contained a considerable amount of X/O active flavoprotein, the material of Mackler *et al.*[8] contained less and that of Morell [4] still less. Further work must aim at the separation of these various types of closely related molecules.

## Experimental

*Xanthine Oxidase Solutions.*—The preparation of the solutions, batch and stage symbols and conditions of storage, etc., together with " AFR," " APR," and " PFR " values for some of the solutions have been recorded.[1, 2]

*Effect of Ammonia on Xanthine Oxidase.*—A sample of batch LXO59, stage M5 (protein concentration approx. 1%), was dialysed at 0—5° in a Visking cellulose bag against a total of 5 l. of aqueous *ca.* 0·01M-ammonia, in five portions, over a period of 10 days. Then the sample was removed, neutralised, centrifuged to remove precipitated material, assayed, and analysed.

" *Inactive* " *Xanthine Oxidase.*—A sample of washings obtained in the preparation of LXO60, M7, was stored at 0—5° for 8 weeks, the activity of the solution in the X/O assay falling from 35 to 0·3 unit/l. The material was precipitated by the addition of ammonium sulphate and phosphate buffer, centrifuged off, redissolved, and dialysed against *ca.* 9 l. of running 0·2M-sodium chloride at 0—5° on a rocking platform. The solution was centrifuged, and the clear supernatant liquor used for assays and analyses.

*Determination of Absorption Coefficient* $E^{1\%}_{1\,cm.}$ (280 mμ) *for Xanthine Oxidase.*—The dry weight of a solution of crystallised xanthine oxidase (LXO55, M7) which had $E^{1\,cm.}_{280}$ 10·0 and $E^{1\,cm.}_{450}$ 1·80 was determined by three methods: (*a*) Kjeldahl nitrogen. Found: N, 1·35 mg./ml. Taking Ball's value [16] for the nitrogen content of crude xanthine oxidase, *viz.*, 16·3% gives the dry weight as 8·3 mg./ml. (*b*) Gravimetric determination with trichloroacetic acid: [17] 9·3 mg./ml.

[14] de Renzo, Heytler, and Kaleita, *Arch. Biochem.*, 1954, **49**, 242.
[15] See also Table 2, sample LXO 60, ex M7.
[16] Ball, *J. Biol. Chem.*, 1939, **128**, 51.

(*c*) An aliquot of the solution was freeze-dried and weighed, and the weight corrected for loss at 110° and for ash weight: Found, 8·5 mg./ml.

The average $E^{1\%}_{1\,cm.}$ value at 280 mμ is therefore 11·5 ± 0·7. Similar figures have been obtained for a number of preparations, including some as crude as M5. The above value differs slightly from that given previously, *viz.*, 11·0 (Avis *et al.*[18]), while the corresponding figure calculated from Ball's data [16] for cruder material ("PFR" = 12·0) is 13·1.

The value of 11·5 has been used in calculating the dry weight from the 280 mμ optical density for all solutions from stage M5 onwards, when these were required for the calculation of composition, etc. It has also been used for cruder samples, when very approximate values would suffice. As the lowest "PFR" so far obtained is 5·0, the value $E^{1\%}_{1\,cm.}$ (450 mμ) at this purity level is 11·5/5·0, *i.e.*, 2·3.

*Fluorimetric Estimations for FAD* (cf. total riboflavin determinations by Burch *et al.*[19]).—Fluorescence was measured relative to an arbitrary eosin reference solution in a Hilger "Spekker" fluorimeter. This eosin solution was introduced as it was found to be more stable than riboflavin solutions of equivalent fluorescence.[20] The FAD concentration was determined by reference to a riboflavin standard (Roche; dried at 110°).

*Microbiological FAD Estimation.*—A sample of LXO56, M7 was assayed microbiologically for riboflavin, after hydrolysis in 0·1N-hydrochloric acid (determination by Messrs. Galloway and Barton-Wright). Under the conditions of the assay, riboflvin and riboflavin phosphate (Roche) gave identical activities on a molar basis. The riboflavin concentration found corresponded to an FAD content of 0·49% (cf. 0·47% by fluorimetry; Table 1, column 3*a*).

*Colorimetric Iron Analyses.*[21]—Aliquot parts were digested with a mixture of perchloric and sulphuric acid, reduced with aqueous sulphur dioxide, and brought to about pH 4 by the addition of excess of sodium acetate, and the colour devloped by adding 2 : 2′-dipyridyl. Readings were taken at 520 mμ, some hours after making up to constant volume.

*Colorimetric Molybdenum Analyses.*—Aliquot parts were digested with perchloric and sulphuric acid, and the colour was developed and measured according to the method of Dick and Bingley.[22]

*Spectrographic Analysis for Metals* (carried out by J. B. Martin).—A dialysed sample (LXO55, M7) was freeze-dried, and the residue was ashed at 435°; spectrographic analysis of the ash gave the following results, expressed as percentages of metal on the original sample: Mo, 0·04—0·05; Fe, 0·18; Cu, 0·004; Pb, 0·004; Ni, 0·003; Mn, 0·0004.* For the corresponding colorimetric values see Table 1 (sample VI, columns 3*b*, *c*).

*Absorption Spectra.*—These were determined in a phosphate buffer of pH 7·1. Freshly prepared LXO60, M7 had maxima at 275, 325, and 454 mμ, minima at 250, 310, and 401 mμ, and light absorption continued up to at least 800 mμ.

*Methylene-blue Assays* (*Reactions* H/Mb *and* A/Mb).—These were carried out by the Thunberg technique, with hypoxanthine or acetaldehyde as substrate, as described by Corran *et al.*[5] Owing to the considerable effect of hypoxanthine concentration on the activity figures (Table 2, footnote *e*) and to the uncertainty of the final volume of solution in the Thunberg tubes after evacuation and flushing with nitrogen, the methylene-blue figures were somewhat less reliable than spectrophotometric assays. For the acetaldehyde figures, the accuracy was further diminished by loss of substrate during evacuation of the tubes. The activities were calculated in the usual manner.

*Spectrophotometric Enzyme Assays.*—These were carried out with a Unicam S.P. 500 Spectrophotometer, at 23·5° ± 0·5° (cf. ref. 1), generally in 1 cm. stoppered cells, in a total volume of 2·6 ml., reaction being started by adding the enzyme last. Optical-density readings at the wavelength selected were taken at intervals, in most cases without a comparison cell, the instrument being balanced in the "check" position against air (cf. ref. 1). When it was desired to reduce readings of high optical density, due, *e.g.*, to the use of turbid solutions, suitable stable, reference liquids, such as solutions of uric acid, sodium dichromate, or ammoniacal copper sulphate, were used for balancing the instrument. For assays on whole milk (Table 2)

* Zn, 0·004 (by colorimetric analysis of LXO73,M7).

[17] Hoch and Vallee, *Analyt. Chem.*, 1953, **25**, 317.
[18] Avis, Bergel, Bray, and Shooter, *Nature*, 1954, **173**, 1230.
[19] Burch, Bessey, and Lowrey, *J. Biol. Chem.*, 1948, **175**, 457.
[20] Bowen and Wokes, "Fluorescence of Solutions," Longmans, Green & Co., London, 1953.
[21] Koenig and Johnson, *J. Biol. Chem.*, 1942, **143**, 159; Sandell, "Colorimetric Determination of Traces of Metals," Interscience Publ., New York, 1950, p. 362.
[22] Dick and Bingley, *Austral. J. Exp. Biol. Med. Sci.*, 1947, **25**, 193.

the validity of the results was checked by adding known amounts of purified enzyme to the milk and repeating the assays. In one experiment in which the activity added was approximately equal to that originally found in the milk, the recoveries of added activity in the X/O and D/I assays were respectively 84 and 72%.

In no reaction were precautions taken to exclude air, and it is assumed that all reaction solutions were therefore saturated (or very nearly saturated) with air at the start of the reaction.

In all the assay systems described below, it was shown that: (1) no significant change of optical density took place if the substrate or the enzyme or the electron-acceptor (where this was not oxygen) was omitted (in the case of some very turbid assay mixtures, changes of optical density due to changing turbidity did occur in the controls, and appropriate corrections to the activities had to be made); (2) the rate of reaction was proportional to the enzyme concentration (see Table 3 for range of rates used). For calculating enzyme activities a general definition of units, similar to that given previously,[1] was adopted: Units/l. $= (dE/dt) \cdot (f/x)$ (where $dE/dt$ is slope at $t = 0$, except for reaction D/I and D/O for which maximum slopes were taken; $f$ = dilution from stock solution to mixture in cell; and $x$ is cell size in cm.). The relative activity of a given enzyme solution in two different assay systems, A and B, was then obtained by:

$$\text{Relative activity} = 100 \times \frac{\text{Units/l. (reaction A)}}{\text{Units/l. (reaction B)}} \times \frac{\Delta\varepsilon \text{ (reaction B)}}{\Delta\varepsilon \text{ (reaction A)}} \%$$

where $\Delta\varepsilon$ is the difference between the molar extinction coefficients of the oxidised and the reduced form. If the component measured in one of the reactions is undergoing single-electron oxidation or reduction, $\Delta\varepsilon$ is replaced by $2\Delta\varepsilon$ for that reaction in the above expression (*e.g.*, in the cytochrome-*c* reaction). When absolute reaction rates were required for reaction X/O, these were calculated as $\mu$ moles/mg. enzyme/min. from $100 \times$ " APR "$E \times {}^{1\%}_{1\text{ cm.}}/\Delta\varepsilon$, where $E^{1\%}_{1\text{ cm.}}$ is the absorption coefficient at 280 m$\mu$ for xanthine oxidase, *i.e.*, 11·5.

*Details of Spectrophotometric Assay Procedures* (see also Table 3).—Buffers: pyrophosphate, pH 8·0, and phosphate, pH 7·1, as used previously [1] for spectrophotometric work.

TABLE 3. *Details of spectrophotometric assay procedures.*

| | | | | Final concentrations, etc. | | | |
|---|---|---|---|---|---|---|---|
| Symbol | $\lambda$ (m$\mu$) | $\Delta\varepsilon$ | pH | Buffer | Substrate concn. ($10^{-4}$M) | Acceptor concn. ($10^{-4}$M) | Range of rates used (1 cm. cell) |
| X/O | 295 | +9,600 | 8·2 | Pyrophosphate (0·05M) | 0·8—1·1 | — | 0·005—0·030/min.[d] |
| X/I | 600 | −21,000 | 8·2 | ,, | 0·8—1·1 | 0·08—0·11 | 0·003—0·050/min. |
| A/I | 600 | −21,000 | 8·2 | ,, | 800 | 0·08—0·11 | 0·003—0·050/min. |
| D/I | 600 | −21,000 | 7·2 | Phosphate (0·03—0·06M) | 0·10—0·14 | 0·08—0·11 | 0·005—0·020/min. |
| D/O | 340 | −6,220[a] | 7·2 | ,, | *ca.* 0·8 | — | 0·002—0·010/min. |
| H/Cy | 550 | +19,600[b] | 7·2 | ,,[c] | 11 | *ca.* 0·4 | 0·002—0·020/min. |

All reactions at 23·5° ± 0·5°, without exclusion of air.

[a] Value from Horecker and Kornberg (*J. Biol. Chem.*, 1948, **175**, 385). [b] Value from Horecker and Heppel.[13a] [c] Reaction mixture contained the following additional components: Ethanol, 0·16M; catalase, *ca.* 7 $\mu$g. of hæmatin/ml.; molybic oxide, $4 \times 10^{-4}$M. [d] For reaction X/O, the initial rate remained strictly proportional to enzyme concentration for rates from 0·001—0·20/min., but for routine determination rates were kept within the range specified.

Reaction X/O: Carried out as described previously.[1]

Reaction X/I: Experiments in which the concentration of 2:6-dichlorophenolindophenol (from B.D.H.) was varied indicated that the quantities given in Table 3 gave maximum activities, with lower activities on increase or decrease of concentration.

Reaction A/I: Acetaldehyde was freshly distilled from paraldehyde and sulphuric acid (older samples which had not deposited crystals were used in some cases); 4% v/v aqueous acetaldehyde was prepared freshly each day and kept cold. (The use of old samples of acetaldehyde appeared to result in slightly low activity figures, and A/I figures are probably less reliable than other assays for this reason.)

Reaction D/I: The stock solution of DPNH (from Sigma material; 0·30—0·35 mg./ml.) deteriorated fairly rapidly at 0—5° (fall in $E_{340}$) and was not kept for more than about 1 week: in this time decomposition was *ca.* 20%. Under the conditions of the assay, the indophenol was decolorised slowly in the absence of enzyme, the rate, $dE/dt$, usually being about −0·0003 per

min. As this rate was always low compared with the enzyme-catalysed rates (see Table 3), no correction was applied. An attempt to use DPNH prepared by reduction of DPN (Light's cozymase) with sodium dithionite [23] was abandoned owing to a very rapid non-enzymic reaction occurring under these conditions. As the unusual features of the D/O reaction (see below) appeared to apply also, to a small extent, to the D/I reaction, maximum $dE/dt$ values, usually attained after 1—5 min., rather than initial values, were recorded, and rates higher than 0·02 or lower than 0·005 per min. were not accepted. After complete reduction of the indophenol, a slow re-oxidation usually occurred in this assay, presumably owing to the presence of dissolved oxygen.

Reaction D/O: For DPNH, either Sigma material or reduced Light's cozymase (see above) was used. Unusual features of the reaction were: (1) slow development of maximum rate of reaction; maximum rather than initial $dE/dt$ values were therefore recorded; (2) fall of specific activities (or units/l.) at high concentrations of enzyme in the assay mixture (cf. Corran *et al.*[12a]); rates higher than 0·01 or lower than 0·002 per min. were not accepted in routine assays.

Reaction H/Cy: Hypoxanthine was from Genatosan; cytochrome-*c* was from Messrs. Light; horse-liver catalase was kindly supplied by Dr. E. F. Hartree; molybdic oxide ("AnalaR") was dissolved in phosphate buffer. The mixture before addition of the enzyme tended to be somewhat turbid, and was therefore generally centrifuged before use. Owing to the very sharp absorption band of reduced cytochrome-*c* at 550 mμ, it was necessary to use a narrow slit width (*ca.* 0·02 mm.) and to check occasionally the setting of the wavelength scale of the instrument as described by the makers. In some cases, the reading corresponding to complete reduction of the cytochrome was determined with sodium dithionite. These readings were not significantly higher than those at the end of the enzymic reductions. In the few experiments performed in the absence of inorganic salts, solutions were dialysed against water before use (cf. Morell [4]).

An experiment under anærobic conditions in an apparatus similar to that of Lazarow and Cooperstein [24] was carried out, the xanthine oxidase being added to the reaction mixture after dissolved air had been removed by bubbling oxygen-free nitrogen through the solution. Xanthine oxidase, LXO64, M5, gave 70 units/l. in the X/O assay. The anærobic H/Cy reaction rate (without catalase and alcohol) corresponded to 0·8 unit/l., compared with 1·2 units/l. obtained under ærobic conditions. In all "ærobic" H/Cy experiments the amount of enzyme used was large, and would probably be sufficient to exhaust the supply of dissolved oxygen (but not the supply of hypoxanthine) in the first few minutes of the run; it seems likely that all rates measured were therefore effectively "anærobic."

*Determination of* $\Delta\varepsilon$ *for Reaction X/O.*—Pure xanthine (Roche Products) was dried at 100°/2 mm. for 2 hr., a solution of known concentration was prepared in the usual buffer mixture, and its absorption measured. The solution (2·6 ml.) was then mixed with a xanthine oxidase concentrate (0·01 ml.), and the absorption was measured again after the rapid reaction had ceased. The molar extinction coefficients for xanthine and uric acid were calculated, after correction for the absorption of the enzyme, for the dilution occurring on addition of the enzyme, and for cell blanks. The values obtained at 295 mμ were: xanthine, $\varepsilon$ 2300; uric acid, $\varepsilon$ 11,900; giving $\Delta\varepsilon$ 9600. The figure for uric acid was confirmed by measurements on a solution prepared from uric acid (from B.D.H.), dried as above: the value obtained differed by less than 1% from that quoted for the enzymically produced uric acid. The $\Delta\varepsilon$ value, which corresponds to $\Delta E$ (1 μg. of xanthine) = 0·063, is probably more reliable than that given previously.[16] Measurements carried out at the same time on the same solutions, at 290 mμ, gave $\Delta\varepsilon$ 7900, *i.e.*, $\Delta E$ (1 μg. xanthine) = 0·052, in reasonable agreement with the value in literature.[25]

*Determination of* $\Delta\varepsilon$ *for Indophenol Reactions.*—A concentrated solution of indophenol was standardised by titration with pure ascorbic acid and, after suitable dilution with phosphate buffer, the optical density at 600 mμ was determined. A control value obtained after reducing with sodium dithionite (cf. reaction H/Cy) was subtracted, and the $\Delta\varepsilon$ value was then calculated directly, giving $\Delta\varepsilon$ 21,000.

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[23] Green and Dewan, *Biochem. J.*, 1937, **31**, 1069.
[24] Lazarow and Cooperstein, *Science*, 1954, **120**, 674.
[25] Krebs and Norris, *Arch. Biochem.*, 1949, **24**, 49.

for metals, and Messrs. Galloway and Barton-Wright have performed the microbiological assay. This investigation and that recorded in Part II were supported by grants to this Institute from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

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## 254. *Tracer Studies in the Formation and Reactions of Organic Per-acids.*

By C. A. BUNTON, (the late) T. A. LEWIS, and D. R. LLEWELLYN.

The acid-catalysed hydrolysis of performic and peracetic acid, and their acid-catalysed formation from the carboxylic acid and hydrogen peroxide, involve acyl–oxygen bond fission. The rate of the acid-catalysed hydrolysis of peracetic acid has been measured. Tracer experiments on the peracetic acid oxidation of benzophenone, 4-methoxybenzophenone, and fluorenone to the corresponding ester or lactone proved that the carbonyl-oxygen of the product came from the ketone and the ethereal oxygen from the per-acid. The course of the reaction is that observed by Doering and Dorfman for the oxidation of benzophenone by perbenzoic acid.

THE hydrolysis of peracetic acid in aqueous perchloric acid at 25·2° was followed by estimation of the per-acid iodometrically, or, as in two preliminary experiments, by following the formation of hydrogen peroxide. The hydrolysis rate was proportional to the acid concentration in the range studied (see Table 1). As the rate follows the concentration of hydroxonium ions, and not the Hammett acidity function $h_0$, we conclude that the transition state includes a water molecule as well as the conjugate acid of peracetic acid.[1] Added sodium perchlorate gives a small decrease in rate, but sodium perchlorate increases the acidity of perchloric acid, as measured by the Hammett acidity function,[2] and therefore should accelerate a unimolecular reaction, whose rate should follow the acidity function.[2] The absence of such an acceleration is therefore additional evidence for a bimolecular reaction.

Qualitative observations were made of the hydrolysis of performic and perbenzoic acid in aqueous perchloric acid at 25·2°. The hydrolysis of performic acid was too fast for convenient measurement since it was not possible to stop the reaction during the decomposition of the hydrogen peroxide with acid ceric sulphate or potassium permanganate. The rate was at least ten times that of the peracetic acid hydrolysis under similar conditions. The hydrolysis of perbenzoic acid was much slower than that of peracetic acid. This rate sequence is qualitatively similar to those observed for the acid hydrolysis of the methyl and ethyl esters of the corresponding carboxylic acids.

TABLE 1. *Rates of hydrolysis of peracetic acid in aqueous perchloric acid at 25·2°.*

| $[HClO_4]$ | 0·022 | 0·096 | 0·310 | 0·483 | 0·886 | 1·57 | 1·82 * | 2·16 | 2·57 | 2·73 | 3·16 † | 3·63 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^5k_1$ (min.$^{-1}$) | 0·14 | 0·75 | 2·05 | 2·90 | 5·68 | 9·7 | 10·0 | 13·6 | 15·9 | 16·6 | 18·3 | 21·3 |
| $10^5k_A$ (mole$^{-1}$ l. min.$^{-1}$) | 6·4 | 7·8 | 6·6 | 6·0 | 6·4 | 6·2 | 5·5 | 6·3 | 6·2 | 6·1 | 5·7 | 5·9 |

* $[NaClO_4] = 0·6$M. † $[NaClO_4] = 0·2$M.

The position of bond fission in the formation and hydrolysis of performic and peracetic acid was determined with $^{18}O$ as a tracer. Isotopically enriched carboxylic acid was treated with 30% hydrogen peroxide in $H_2{}^{18}O$ (in the presence of sulphuric or perchloric acid) at room temperature. When equilibrium was attained the solutions were neutralised, hydrogen peroxide pumped off, and the peracid hydrolysed in $H_2{}^{18}O$; the hydrogen peroxide so formed was found to be isotopically normal. The isotopic analyses of the hydrogen

[1] Hammett, "Physical Organic Chemistry," McGraw-Hill Book Company Inc., New York, 1940, p. 267; Bell, Dowding, and Noble, *J.*, 1955, 3106.
[2] Long and McIntyre, *J. Amer. Chem. Soc.*, 1954, **76**, 3243.

peroxide were carried out by decomposition with acid potassium permanganate and mass-spectrometric analysis of the evolved oxygen. In this decomposition it has been shown that the oxygen is derived from the hydrogen peroxide.

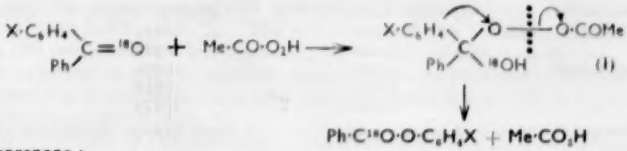
The only interpretation of this isotopic and kinetic evidence is that at no stage in formation or hydrolysis of a per-acid is the peroxide bond broken, and that the mechanism is analogous to the $A_{Ac}2$ mechanism of carboxyl esterification and hydrolysis: [3]

$$R{\cdot}CO{\cdot}O_2H_2^+ + H_2{}^{18}O \longrightarrow R{\cdot}C^{18}O{\cdot}OH_2^+ + H_2O_2$$

(In this formulation the position of the proton is assumed.)

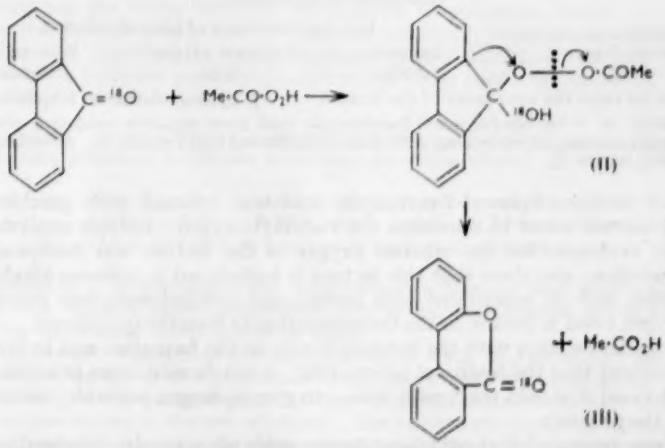
The oxidation of benzophenone by perbenzoic acid in benzene was examined by Doering and Dorfman,[4] whose findings are in accord with the general mechanism of Criegee [5] and Robertson and Waters.[6] Mechanisms in which either the oxygen atoms of the ester became equivalent, or the per-acid formed the hydroxyl cation, $OH^+$, were excluded. It seemed possible that in a more ionizing solvent, and in the presence of mineral acid, other mechanisms might be observed. The oxidations of benzophenone and 4-methoxybenzophenone in acetic acid solution by peracetic acid in the presence of sulphuric acid were examined isotopically, and it was found that the ethereal oxygen was derived from the per-acid,[7] as required by the mechanism of Criegee [5] and Robertson and Waters.[6]

These observations have now been extended to the oxidation of fluorenone to give the lactone of 2′-hydroxydiphenyl-2-carboxylic acid (III). The reactions are shown in the annexed scheme. The oxidation of ketones by per-acids is acid-catalysed, as are many



$$Ph{\cdot}C^{18}O{\cdot}O{\cdot}C_6H_4X + Me{\cdot}CO_2H$$

For fluorenone :



carbonyl-addition reactions. For simplicity the proton has been omitted in the formulations in the scheme : it is presumably located on the carbonyl-oxygen atom of the ketone.

The oxygen of the ketone and the acetic acid was isotopically enriched, and after

[3] Day and Ingold, *Trans. Faraday Soc.*, 1941, **37**, 686.
[4] Doering and Dorfman, *J. Amer. Chem. Soc.*, 1953, **75**, 5595.
[5] Criegee, *Ann. Chim. (France)*, 1947, **127**, 560.
[6] Robertson and Waters, *J.*, 1948, 1574.
[7] Bunton, Lewis, and Llewellyn, *Chem. and Ind.*, 1954, 191.

reaction the oxygen isotopic content of the product ester or lactone was determined. This was done for phenyl benzoate and *p*-methoxyphenyl benzoate by alkaline hydrolysis in isotopically normal water. Independent experiments proved that phenyl benzoate undergoes acyl–oxygen fission under these conditions. Thus, to a first approximation, one oxygen atom of the isolated benzoic acid is the carbonyl-oxygen atom of the ester (the other coming from the water), and the oxygen of the phenol is the ethereal oxygen of the ester.

The results for the $^{18}O$ abundance of the ester are given in Table 2. In calculation of the results, isotopic equilibration between the oxygen atoms of the ketone, acetic acid, and water under the strongly acidic conditions of the oxidation is assumed, and isotope dilution by the acetic acid formed in the course of the oxidation is ignored. This dilution causes the isotopic abundance of the carbonyl-oxygen of the ester to be lower than that calculated for the ketone.

The oxidation of fluorenone was studied by a similar method. The isotopic abundance of the lactone was determined by pyrolysis to carbon monoxide. The lactone was hydrolysed and methylated in $H_2{}^{18}O$ to give 2′-methoxydiphenyl-2-carboxylic acid, and this acid converted into 2-hydroxydiphenyl by heating it with soda-lime. Isotopic analysis proved that the hydroxyl-oxygen, and hence the ethereal oxygen of the lactone, was isotopically normal, as is found for per-acid oxidation of open-chain ketones. Another

TABLE 2. *Oxidation of open-chain ketones.*

| Compound | Calc. isotopic abundance of ketone (atom % $^{18}O$) | Isotopic abundance of product (atom %) | |
|---|---|---|---|
| | | Carbonyl oxygen * | Ethereal oxygen † |
| Benzophenone ..... | 0·586 | 0·514 | 0·001 |
| ,, ..... | 0·630 | 0·474 | 0·002 |
| 4-Methoxybenzophenone ..... | 0·720 | 0·560 | 0·004 |
| ,, ..... | 0·605 | 0·610 | 0·002 |
| ,, ..... | 0·450 | 0·374 | 0·005 |

* From isotopic abundance of benzoic acid. † From isotopic abundance of phenol.

*Oxidation of fluorenone.*

| Calc. isotopic abundance of ketone (atom %) | Isotopic abundance of product (atom %) | | |
|---|---|---|---|
| | Lactone | Carbonyl oxygen * | Ethereal oxygen † |
| 0·60 | 0·275 | 0·55 | 0·025% |

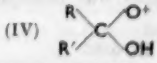
* Taken to be twice the abundance of the lactone. † By degradation to 2-hydroxydiphenyl.

Abundance of 2′-methoxydiphenyl-2-carboxylic acid from separate oxidation with carboxyl-oxygen atoms normalised by boiling with water: 0·039 and 0·043 atoms %. Abundance of original ketone 0·95% atoms %.

sample of 2′-methoxydiphenyl-2-carboxylic acid was refluxed with perchloric acid in isotopically normal water to normalise the carboxyl-oxygen; isotopic analysis then gave independent evidence that the ethereal oxygen of the lactone was isotopically normal. These degradations also show that this lactone is hydrolysed in aqueous alkali with acyl–oxygen fission, and an experiment with isotopically enriched soda-lime proves that the methyl–oxygen bond is broken in the demethylation of 2-methoxydiphenyl.

These results, together with the isotopic results on the formation and hydrolysis of the per-acids, suggest that the hydroxyl cation, $OH^+$, is not formed, even in acidic conditions. If it were formed it should react with water, to give hydrogen peroxide, and so introduce tracer into the peroxide.

It has been suggested that oxidations by per-acids often involve intermediates containing cationic oxygen,[9] and that the intermediates [(I) and (II)], formed by per-acid oxidation at the carbonyl-carbon atom, give entities of the general formula (IV), which then rearrange to the product, thus:

(IV)  $\longrightarrow$ $R'{\cdot}CO_2R + H^+$

[8] Friess and Soloway, *J. Amer. Chem. Soc.*, 1951, **73**, 3968.
[9] Leffler, *Chem. Rev.*, 1949, **45**, 385.

If such an intermediate (IV) involving cationic oxygen were formed, and had an appreciable life, the oxygen atoms could become equivalent by the ready hydrogen transfer. The fact that they do not, even in a good ionizing solvent, suggests that the intermediate (IV), if formed, has a very short life, or that the breaking of the oxygen–oxygen bond is synchronous with, and assisted by, the migration.[4, 10]

## Experimental

Results of typical experiments on the formation and hydrolysis of per-acids are given below. All isotopic abundances are in atom % excess above normal.

*Preparations.—Isotopically enriched acetic acid.* Acetic anhydride (34 g.) was hydrolysed by 6 c.c. of $H_2{}^{18}O$ (abundance 2·72 atom %). Calculated abundance of acetic acid, 0·68 atom %.

*Isotopically enriched formic acid.* Anhydrous formic acid (12 g.) was left to equilibrate with 1·5 c.c. of $H_2{}^{18}O$ (abundance 9·1 atom %). Calculated abundance of formic acid 1·25 atom %.

*Formation and Hydrolysis of Per-acids.*—Enriched acetic acid was treated with 2 drops of concentrated sulphuric acid and 2 c.c. of 90% hydrogen peroxide. After 6 hr. at 20° the per-acetic acid contained *ca.* 65% of total peroxide. The solution was diluted with 100 c.c. of 0·5 atom % $H_2{}^{18}O$, and the pH adjusted to 9. The liquid was then frozen, and hydrogen peroxide pumped off and decomposed with potassium permanganate. The abundance of the evolved oxygen was 0·004 atom %. The solution was then acidified, and the per-acid hydrolysed; the hydrogen peroxide had an oxygen isotopic abundance of −0·001 atom %.

The experiment with enriched formic acid was conducted in a similar manner, but on a smaller scale. The isotopic abundance of the residual hydrogen peroxide (after formation of the performic acid) was 0·002 atom %. The abundance of the hydrogen peroxide produced by hydrolysis of this performic acid in 0·5 atom % $H_2{}^{18}O$ was −0·002 atom %.

Duplicate experiments gave isotopically normal hydrogen peroxide in all cases.

An experiment in which perphthalic acid was hydrolysed in 0·5 atom % $H_2{}^{18}O$ gave hydrogen peroxide of abundance 0·002 atom %.

*Kinetics of Hydrolysis of Peracetic acid.*—The hydrolyses were followed in aqueous perchloric acid by first titrating the hydrogen peroxide with potassium permanganate or ceric sulphate, and then titrating the iodine liberated on addition of potassium iodide. In general the integrated first-order rate constants were calculated graphically from the peracetic acid concentrations. The rate constants of two preliminary runs ($[HClO_4]$ = 2·2M and 2·57M) were calculated from the hydrogen peroxide concentrations. The results are quoted in Table 1, and the data of a kinetic run are annexed.

$[HClO_4]$ = 2·16M. Portions (5 c.c.) were titrated with 0·0327N-potassium permanganate to destroy hydrogen peroxide, potassium iodide was added, and the iodine titrated with 0·0317N-sodium thiosulphate:

| Time (min.) ..... | 0 | 5 | 10 | 20 | 30 | 40 | 50 | 60 | 70 |
|---|---|---|---|---|---|---|---|---|---|
| $Na_2S_2O_3$ (c.c.) ..... | 6·63 | 6·44 | 6·20 | 5·34 | 4·64 | 3·90 | 3·54 | 3·10 | 2·74 |

$k_1 = 13{\cdot}6 \times 10^{-4}$ (min.$^{-1}$) from plot of log $(a - x)$ against time.

*Ketone Oxidation.*—Benzophenone and 4-methoxybenzophenone were isotopically enriched by refluxing with acidified dioxan and $H_2{}^{18}O$. The isotopic abundances of the ketones were calculated from the isotopic abundance of the water.

The ketone was left overnight in isotopically enriched acetic acid containing sulphuric acid. This mixture was then added to a solution of isotopically normal peracetic acid in isotopically enriched acetic acid, of a composition such that the $^{18}O$ abundances of the ketonic and carboxylic oxygen atoms were similar in the two solutions. The isotopic abundance of the oxygen of the ketone was calculated by assuming complete isotopic equilibriation between the ketone and the acetic acid solvent. To test this assumption, some experiments were carried out with isotopically normal ketone added directly to the solution of peracetic acid in isotopically enriched acetic acid containing sulphuric acid. The solutions were then left at room temperatures for several days (the general reaction conditions of Doering and Spears [11]). The product was isolated by pouring the mixture into water, filtering, and hydrolysing the crude ester with sodium hydroxide in isotopically normal water. The oxygen isotopic abundance of the phenol

[10] Friess, *J. Amer. Chem. Soc.*, 1949, **71**, 2571.
[11] Doering and Spears, *ibid.*, 1950, **72**, 5515.

was determined by pyrolysis *in vacuo* to carbon monoxide on heated metal gauze, or on red-hot carbon. As a test of the analytical method, some carbon monoxide samples were converted catalytically into carbon dioxide, and this gas was analysed mass-spectrometrically. The benzoic acid was converted into its silver salt, and this decarboxylated by heat *in vacuo*. All isotopic analyses were carried out mass-spectrometrically.

The lactone (III) of 2′-hydroxydiphenyl-2-carboxylic acid was isolated from oxidation of fluorenone with peracetic acid, and a portion pyrolysed to carbon monoxide. Another portion was converted into 2′-methoxydiphenyl-2-carboxylic acid by hydrolysis in $H_2{}^{18}O$ (abundance, *ca.* ½ atom %), and methylation with dimethyl sulphate.[12] This acid was then decarboxylated and demethylated by heating it with soda-lime, and the 2-hydroxydiphenyl so formed was pyrolysed to carbon monoxide and shown to be isotopically normal in oxygen. The product of another oxidation of fluorenone was similarly converted into 2′-methoxydiphenyl-2-carboxylic acid, and this acid heated with aqueous perchloric acid to remove any tracer in the carboxyl-oxygen atoms; it was assumed that the methoxyl oxygen was not affected by this treatment. The isotopic abundance of this " normalised " acid was determined by pyrolysis to carbon monoxide.

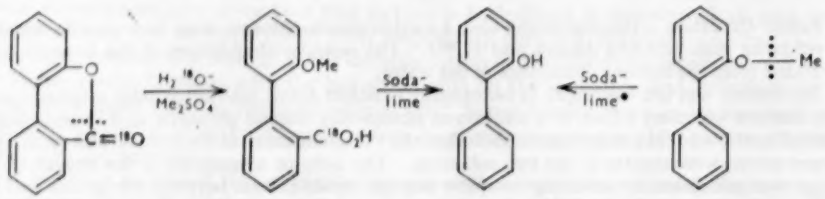
Examples of the oxidations and degradations are given below.

*Benzophenone.* Acetic anhydride (0·246 mole) was hydrolysed in 0·25 mole of $H_2{}^{18}O$ (abundance 2·80 atom %). A portion was added to 0·033 mole of benzophenone (abundance 1·61 atom %) with 7 c.c. of concentrated sulphuric acid, and the residue to 12 g. of 40% peracetic acid in acetic acid. The following morning the solutions were mixed and left for 4 days at room temperature. The phenyl benzoate gave phenol (abundance 0·001 atom %) and benzoic acid (abundance 0·257 atom %); the abundance calculated from that of the ketone was 0·293 atom %.

*Fluorenone.* Acetic anhydride (0·5 mole) was hydrolysed in 0·5 mole of $H_2{}^{18}O$ (abundance 2·80 atom %), and fluorenone (6 g.) and 10 c.c. of concentrated sulphuric acid were then added, followed by 22 g. of 40% peracetic acid in acetic acid. The solution was left at room temperature for 10 days and then poured into 250 c.c. of $H_2{}^{18}O$ (0·4 atom %). Some of the lactone was isolated, and purified (m. p. 91—92°, isotopic abundance 0·275 atom %, isotopic abundance calculated from isotopic abundance of ketone 0·30 atom %). The remainder of the lactone was hydrolysed and methylated with dimethyl sulphate in $H_2{}^{18}O$. The 2′-methoxydiphenyl-2-carboxylic acid so formed was heated with soda-lime to give 2-hydroxydiphenyl (m. p. 54—56°; isotopic abundance 0·025 atom %).

A similar oxidation with peracetic acid (formed by the reaction of acetic anhydride and 90% hydrogen peroxide) in isotopically enriched acetic acid gave a lactone which, after hydrolysis and methylation in $H_2{}^{18}O$ (*ca.* 0·5 atom % abundance), gave 2′-methoxydiphenyl-2-carboxylic acid (m. p. 152°).

After being heated for several days in 2N-perchloric acid, the 2′-methoxydiphenyl-2-carboxylic acid had m. p. 151—152°, and isotopic abundance 0·039 and 0·043 atom % (separate determinations). Demethylation of 2-methoxydiphenyl by isotopically enriched soda-lime (isotopic abundance *ca.* 0·3 atom %) gave 2-hydroxydiphenyl (m. p. 53—55°, isotopic abundance 0·003 atom %). The reactions can be represented by the annexed scheme.




(Abundance, 0·275 atoms %) (Isotopically normal) * Abundance *ca.* 0·3 atom %

The authors are indebted to Professors C. K. Ingold, F.R.S., and E. D. Hughes, F.R.S., for their valuable advice on this work.

Sir William Ramsay and Ralph Forster Laboratories,
University College, London. [*Received, September 6th*, 1955.]

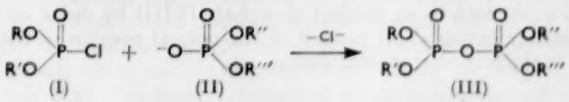
[12] Rule and Bretscher, *J.*, 1927, 925.

## 255. *Studies on Phosphorylation. Part XIII.* Ketoxime Sulphonates as Intermediates in Pyrophosphate Formation.*

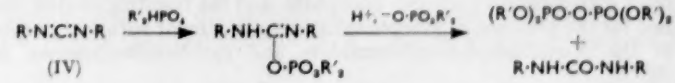
By G. W. Kenner, Sir Alexander R. Todd, and R. F. Webb.

Imidoyl phosphates (VIII) have been prepared by the Beckmann rearrangement of the arylsulphonyl derivatives (VI) of ketoximes in the presence of phosphate anions, and have been used for the preparation of pyrophosphates. These reactions constitute a general method likely to be useful in the coenzyme field.

Of the methods developed in the present series for the preparation of polyphosphates that which involves the reaction between a phosphorochloridate (I) and the salt of a phosphoric acid (II) has hitherto been the most widely used in the nucleotide series.[1-4] This method and those employing exchange reactions involving tetraphenyl pyrophosphate or trifluoroacetic anhydride [5,6] are, however, subject to the limitation that the sugar- and/or phosphate-hydroxyl residues in reactive nucleoside intermediates must be shielded during the condensation by hydrophobic protecting groups which are subsequently removed. The extreme lability of the fully esterified condensation products, and of many of the nucleotide coenzymes themselves, to both acid and alkali has stimulated the development of synthetic methods in which the requisite nucleotide intermediates do not require the usual protecting groups since the conditions under which these groups can be removed are often those which cause extensive destruction of the desired product.




The introduction of the carbodi-imide method [7] for the synthesis of $P^1P^2$-diesters of pyrophosphoric acid (III; R = R'' = H) from free monoalkyl phosphates or their pyridine salts, together with the demonstration that neither traces of water [7] nor certain free sugar-hydroxyl groups [6] seriously interfered with pyrophosphate formation, appeared to offer a route to many nucleotide coenzymes and it has since been used in the synthesis of uridine-diphosphate-glucose,[8] adenosine-5′ triphosphate,[9] and uridine-5′ triphosphate.[10] The formation of pyrophosphates by the interaction of carbodi-imides and phosphoric acids was represented [7] as proceeding by initial addition of the acid to the carbodi-imide (IV) followed by phosphorolysis of the adduct:

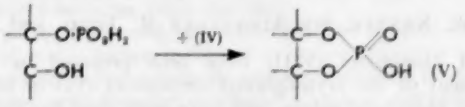



Isolation of the intermediate adduct was not possible, with the result that the coupling of two different phosphoric acids invariably gave mixtures of the two symmetrical pyrophosphates together with the unsymmetrical product, thus greatly complicating the isolation procedures.[8]

* Part XII, *J.*, 1954, 2381.

[1] Baddiley and Todd, *J.*, 1947, 648.
[2] Baddiley, Michelson, and Todd, *J.*, 1949, 582; Michelson and Todd, *ibid.*, p. 2487.
[3] Kenner, Todd, and Weymouth, *J.*, 1952, 3675; Kenner, Todd, Webb, and Weymouth, *J.*, 1954, 2288.
[4] Christie, Kenner, and Todd, *ibid.*, p. 46.
[5] Corby, Kenner, and Todd, *J.*, 1952, 1234.
[6] Christie, Elmore, Kenner, Todd, and Weymouth, *J.*, 1953, 2947.
[7] Khorana and Todd, *ibid.*, p. 2257.
[8] Kenner, Todd, and Webb, *J.*, 1954, 2843.
[9] Khorana, *J. Amer. Chem. Soc.*, 1954, **76**, 3517.
[10] Hall and Khorana, *ibid.*, p. 5066.

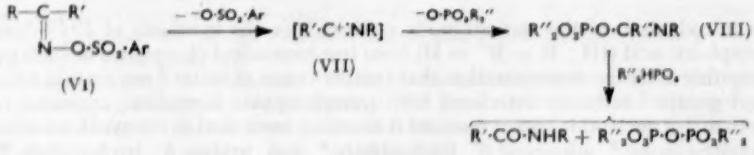
A further complication arises when carbodi-imides are used to form pyrophosphates from esters of phosphoric acid bearing a hydroxyl group in a vicinal position to the phosphate since here cyclic phosphate (V) formation may predominate: [8]




Finally, neither trialkylammonium nor tetra-alkylammonium salts of phosphates, which may have convenient solubilities in organic solvents, can be used in the carbodi-imide process.

In attempts to overcome some of these restrictions, we investigated the use of imidoyl phosphates. Dr. F. R. Atherton had already shown that dibenzyl *N*-phenylbenzimidoyl phosphate (VIII; R = R′ = Ph, R″ = $CH_2Ph$) undergoes phosphorolysis when treated with either dibenzyl or diphenyl hydrogen phosphate, yielding the pyrophosphate and benzanilide; [11] this reaction is analogous to the hypothetical second step in the carbodi-imide reaction sequence.

The imidoyl phosphate (VIII; R = R′ = Ph, R″ = $CH_2Ph$) was prepared by Dr. Atherton from *N*-phenylbenzimidoyl chloride and silver dibenzyl phosphate. It occurred to us that a wider series of imidoyl phosphates might be made accessible by the Beckmann rearrangement of oximes. The current view is that the first step of rearrangement in non-hydroxylic media is ionisation with simultaneous intramolecular alkyl or aryl migration, yielding an ion such as (VII). Consequently rearrangement in presence of a phosphate anion could lead to an imidoyl phosphate (VIII) by union of the added anion with the intermediate cation (VII) instead of the normal reunion of the latter with the anion generated in the first rearrangement step.




In a preliminary experiment it was demonstrated that the diphenyl phosphoryl derivative of acetoxime gave the imidoyl phosphate (VIII; R = R′ = Me, R″ = Ph) by straightforward Beckmann rearrangement during distillation at 120°. The product was allowed to react with dibenzyl hydrogen phosphate, and the resulting neutral material was then examined for the presence of pyrophosphates by treatment with *cyclo*hexylamine and detection of the *N-cyclo*hexylphosphoramidate and *cyclo*hexylammonium phosphates (cf. Part X [5]).

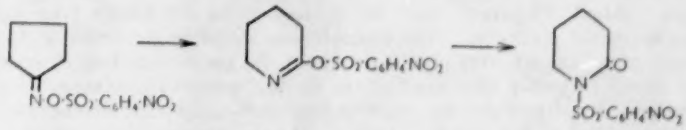
As starting materials for the synthetic method proper the arenesulphonyl derivatives of ketoximes (VI) were selected, since the arenesulphonate ion should not compete effectively with the added phosphate ion for the intermediate cation (VII) and a series of these compounds had been described by Oxley and Short [12] who had used them for the preparation of amidines. In the present investigation the ketoxime sulphonates required were those which could readily be obtained pure, could be stored without spontaneous rearrangement, and would rearrange at a suitable rate in a polar solvent. The suitability of various oxime esters in the synthesis of tetrabenzyl pyrophosphate was determined by reaction of the oxime ester (1 mol.) in a suitable solvent with dibenzyl hydrogen phosphate (2 mols.) in the presence of various tertiary amines (1 mol.). The yield of tetrabenzyl pyrophosphate produced in each experiment was estimated by the *cyclo*hexylamine assay previously

[11] Atherton, personal communication; Atherton, Morrison, Cremlyn, Kenner, Todd, and Webb, *Chem. and Ind.*, 1955, 1183.
[12] Oxley and Short, *J.*, 1948, 1514.

described, the yield of the tetraester produced being based on the weight of *cyclo*hexylamine salt isolated. The validity of this procedure was checked in certain cases by subsequent isolation of the *N-cyclo*hexylphosphoramidate also produced in the *cyclo*hexylamine treatment and also by isolation of tetrabenzyl pyrophosphate in separate experiments. The results may be summarised as follows.

As previously shown by Oxley and Short,[12] acetoxime benzenesulphonate did not undergo the Beckmann rearrangement except at elevated temperature and was therefore unsuitable for the present study. Acetophenone oxime toluene-*p*-sulphonate could not be obtained pure since the solid ester rearranged rapidly at room temperature. The crude material, however, gave some tetrabenzyl pyrophosphate (16%) when allowed to react with dibenzyl hydrogen phosphate and triethylamine in benzene at 18° for 16 hr. The use of a more polar solvent, methyl cyanide, under the same conditions doubled the yield of pyrophosphate. Ethyl methyl ketoxime benzenesulphonate also gave fair yields (20—38%) of tetrabenzyl pyrophosphate under various conditions; the use of methyl cyanide as solvent again improved yields of the pyrophosphate in comparison with benzene. The use of 2 : 6-lutidine in place of triethylamine as base had no marked effect on yields. *cyclo*Pentanone oxime benzenesulphonate was prepared as a low-melting solid but the material could be stored at −40° without spontaneous rearrangement. The rate of rearrangement of the compound in chloroform and methyl cyanide was determined. In chloroform rearrangement was slow (about 25% in 200 min. at 25°); in methyl cyanide it was virtually complete in 95 min. at 25°. However, in the presence of 2 : 6-lutidine (1 mol.) and dibenzyl hydrogen phosphate (2 mols.) in methyl cyanide at 18° for 16 hr. only a 40% yield of tetrabenzyl pyrophosphate was obtained, probably owing to some inhibition by this base. Markedly more pyrophosphate was obtained when nitromethane replaced methyl cyanide as solvent. *cyclo*Pentanone oxime *p*-nitrobenzenesulphonate was found to be the most suitable reagent for the synthesis of tetrabenzyl pyrophosphate, yields up to 90% being obtained with nitromethane as solvent at room temperature; the reagent, moreover, can be stored indefinitely at 0°. In methyl cyanide at 25° its rearrangement was complete in 50 min., whereas in chloroform only 40% of the ester had rearranged after 200 min. Comparison of these rates, together with those for the corresponding benzenesulphonates reveals the well-recognised influences of acid strength in the oxime ester and polarity of the solvent on the rate of the Beckmann rearrangement.[13,14]

Another possible route to imidoyl phosphates (VIII) involves phosphate–sulphonate anion-exchange on an imidoyl sulphonate which may itself be produced by the Beckmann rearrangement of a ketoxime sulphonate.[13] In the present investigation only a preliminary examination of imidoyl sulphonates was undertaken; a more detailed consideration will be presented in a later paper. *cyclo*Pentanone oxime *p*-nitrobenzenesulphonate rearranged spontaneously in nitromethane to the enol *p*-nitrobenzenesulphonate (IX) of valerolactam which was then treated with dibenzyl hydrogen phosphate (2 mols.) and triethylamine (1 mol.). The yield of the pyrophosphate (27%; assayed as above) was much lower than

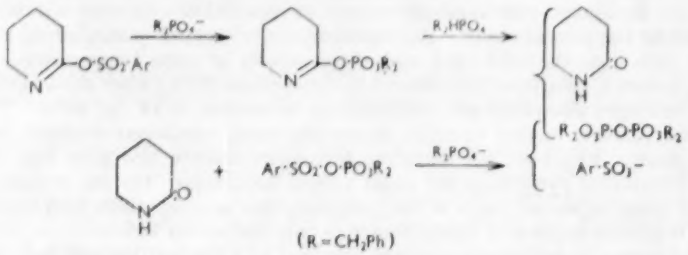



(IX) (X)

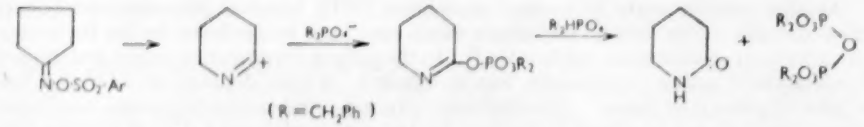
when the phosphate was present during the step involving Beckmann rearrangement (80%), under otherwise standard conditions. Further, the reaction involving the imidoyl sulphonate, in contrast to that with the ketoxime sulphonate, was apparently base-catalysed since an increase in the proportion of triethylamine (to 3 mols.) in the above experiment

[13] Kuhara, "The Beckmann Rearrangement," Komatsu, Tokyo, 1926.
[14] Chapman, *J.*, 1934, 1550.

raised the yield of tetrabenzyl pyrophosphate (to 44%). The lower yield of tetrabenzyl pyrophosphate in the reaction involving the preformed imidoyl sulphonate was not due to further rearrangement of the imidoyl sulphonate to the *N*-acylsulphonamide (X), *i.e.*, Chapman change,[15] since the Chapman change is itself probably base-catalysed (cf. the use of pyridine in preparation of *N*-acylureas from carbodi-imides and carboxylic acids [16]) and




also since the imidoyl sulphonate solution, even after 20 hr. at room temperature, still liberated 1 mol. of *p*-nitrobenzenesulphonic acid when treated with water; the *N*-acylsulphonamides would not liberate acid under these conditions. Also, the choice of *cyclo*pentanone oxime esters for the rearrangement largely precludes any intramolecular sulphonyl or phosphoryl migration in the piperidone derivative (cf. the effect of ring size on the analogous rearrangement of *N*-nitrosolactams [17]). We therefore consider that two possible mechanisms may account for the formation of pyrophosphates from imidoyl sulphonates, one involving sulphonate–phosphate anion-exchange followed by a phosphorolytic step; the other involving the formation of a mixed anhydride between *p*-nitrobenzenesulphonic and dibenzyl phosphoric acids which may then undergo phosphate exchange (cf. Part X [5]). We further conclude that this reaction differs from that involving the ketoxime sulphonate where the exchange of anions occurs during the Beckmann rearrangement, thus affording further evidence for the intermolecular migration of the anion in the Beckmann rearrangement of ketoxime esters (cf. Brodskii and Miklukin [18]):




Having established the reaction conditions and the preferred ketoxime ester we investigated the synthesis of an unsymmetrical pyrophosphate derivative. In the synthesis of tetrabenzyl pyrophosphate from trialkylammonium dibenzyl phosphates no intermediate imidoyl phosphate could be isolated. The Beckmann rearrangement of *cyclo*pentanone oxime *p*-nitrobenzenesulphonate was therefore conducted in the presence of tetraethylammonium dibenzyl phosphate; at this stage no tetrabenzyl pyrophosphate had been formed. Further treatment of the imidoyl phosphate solution with diphenyl hydrogen phosphate, with or without added tertiary amine, in a polar solvent (nitromethane) gave a mixture of pyrophosphates as judged by the *cyclo*hexylamine assay. However, in a non-polar solvent (benzene), the unsymmetrical pyrophosphate, dibenzyl diphenyl pyrophosphate, was formed almost exclusively. It is apparent from this, and from work to be reported later, that in a polar solvent the imidoyl phosphate may undergo two competing reactions, namely, phosphorolysis to the pyrophosphate and the amide, and exchange of phosphate leading ultimately to mixtures of pyrophosphates.

[15] Chapman and Howis, *J.*, 1933, **806**.
[16] Zetsche and Fredrich, *Ber.*, 1939, **72**, 1735.
[17] Huisgen and Reinertshofer, *Annalen*, 1952, **575**, 197.
[18] Brodskii and Miklukin, *Compt. rend. Acad. Sci. U.R.S.S.*, 1941, **32**, 588.

The method outlined above employing ketoxime sulphonates as intermediates in the formation of tetraesters of pyrophosphoric acid is therefore complementary to that employing phosphorochloridates [19] in non-polar media; however, in polar media the products are similar to those obtained by the use of carbodi-imides (Part XI [7]).

## Experimental

*Diphenyl Phosphorylation of Acetoxime and Partial Thermal Rearrangement of the Product.*—Acetoxime (3·4 g.) in pyridine (4 c.c.) at 0° was treated with diphenyl phosphorochloridate [20] (11·6 g.) in pyridine (20 c.c.). The solution was kept at 0° for 18 hr., then poured into ice-water (150 c.c.), the precipitated oil was collected and dissolved in chloroform (20 c.c.), and the solution washed with saturated potassium hydrogen carbonate solution, water, saturated sodium hydrogen carbonate solution, and water. The dried ($Na_2SO_4$) solution was evaporated *in vacuo* and the residual oil (7·76 g., 72%) distilled in small portions (*ca.* 2 g.) when required, affording a colourless oil, b. p. 120° (bath-temp.)/1·0 × $10^{-4}$ mm., which was a mixture of acetoxime diphenyl phosphate and diphenyl *N*-methylacetimidoyl phosphate as shown by the following experiment.

*Pyrophosphate formation from the above crude product.* A solution of the crude product (0·5 g.) and dibenzyl hydrogen phosphate (0·52 g., 1·1 mol.) in dry carbon tetrachloride (2 c.c.) was refluxed for 4 hr., washed with saturated sodium hydrogen carbonate solution then with water, dried ($CaCl_2$), and evaporated *in vacuo*. The residue was shown to contain pyrophosphates by treatment with *cyclo*hexylamine (Part X [5]) as follows. The residue was dissolved in ethyl methyl ketone (5 c.c.), the solution evaporated under reduced pressure, the residue redissolved in ethyl methyl ketone (5 c.c.), *cyclo*hexylamine (1 c.c.) added, and the mixture kept at 20° for 16 hr. The precipitate (0·1 g., 20%) was filtered off, washed with benzene (2 c.c.) and ether (2 c.c.), and when dried *in vacuo* over phosphoric oxide had m. p. 174—182°. Recrystallisation (twice) from ethanol afforded *cyclo*hexylammonium diphenyl phosphate (0·04 g.), m. p. and mixed m. p. 199—200°. The filtrate and washings from the *cyclo*hexylamine fission were evaporated *in vacuo*, the residue was dissolved in benzene (4 c.c.) and washed with N-hydrochloric acid (3 × 4 c.c.), water (8 c.c.), saturated sodium hydrogen carbonate solution (2 × 4 c.c.), and again with water (2 × 4 c.c.). The dried ($Na_2SO_4$) solution was evaporated *in vacuo* and the semi-solid residue (0·19 g.) pressed on a porous tile. The solid residue (0·11 g., 20%) had m. p. 68—70°, and after crystallisation from *cyclo*hexane gave dibenzyl *N*-cyclohexylphosphoramidate (0·06 g.), m. p. and mixed m. p. 78—80°.

*Preparation of Ketoxime Sulphonates.*—The ketoxime sulphonates were prepared by the general method of Oxley and Short,[13] as illustrated by the following.

*cyclo*Pentanone (4·2 g.) was added dropwise to a stirred solution of hydroxylammonium chloride (3·5 g., 1 mol.) in aqueous 4·5N-potassium hydroxide (25 c.c.) at 0°. The solution was stirred at 0° for a further 40 min. and then a solution of *p*-nitrobenzenesulphonyl chloride [21] (11·1 g., 1·02 mol.) in acetone (35 c.c.) was slowly added, the mixture being kept at 0—5°. The mixture was stirred at 0—5° for a further 30 min., the precipitate filtered off, washed with ice-cold aqueous potassium hydroxide (50 c.c. of 5%) and with ice-cold water (100 c.c.), and the solution dried ($K_2CO_3$), filtered, and evaporated *in vacuo* at room temperature until solid began to separate. Light petroleum (110 c.c.; b. p. 60—80°) was added, and the mixture set aside at 0° for 30 min. and filtered. Recrystallisation from chloroform–light petroleum (b. p. 60—80°) gave cyclo*pentanone oxime* p-*nitrobenzenesulphonate* (11·0 g., 77%), m. p. 69° (decomp.) (Found: C, 46·5; H, 4·1; N, 9·8. $C_{11}H_{12}O_5N_2S$ requires C, 46·5; H, 4·3; N, 9·9%).

Similarly prepared were acetoxime benzenesulphonate,[22] m. p. 53°, acetophenone oxime toluene-*p*-sulphonate,[13] m. p. *ca.* 30°, ethyl methyl ketoxime benzenesulphonate,[23] m. p. 48° (Found: C, 52·4; H, 5·7. Calc. for $C_{10}H_{13}O_3NS$: C, 52·8; H, 5·8%), and *cyclo*pentanone oxime benzenesulphonate [13] (Found: C, 54·0; H, 5·8; N, 6·1%; sap. equiv., 241. Calc. for $C_{11}H_{13}O_3NS$: C, 55·2; H, 5·5; N, 5·9%; equiv., 239).

*Rate of Rearrangement of* cyclo*Pentanone Oxime Sulphonate.*—0·2M-Solutions of *cyclo*pentanone oxime benzenesulphonate and *p*-nitrobenzenesulphonate in methyl cyanide (10 c.c.) and chloroform (10 c.c.) were kept at 25°. Aliquot parts (1 c.c.) were withdrawn at intervals

[19] Toy, *J. Amer. Chem. Soc.*, 1950, **72**, 2065.
[20] Brigl and Müller, *Ber.*, 1939, **72**, 2121.
[21] Barber, *J.*, 1943, 102.
[22] Wege, *Ber.*, 1891, **24**, 3538.

and added to excess of 0·1N-sodium hydroxide, the mixtures diluted with water, and the excesses of alkali back-titrated with 0·0911N-hydrochloric acid. The results are tabulated.

| Solvent | cyclo*Pentanone oxime benzenesulphonate* | | cyclo*Pentanone oxime* p-*nitrobenzenesulphonate* | |
|---|---|---|---|---|
| | Time (min.) | Rearrangement (%) | Time (min.) | Rearrangement (%) |
| $CHCl_3$ | 90 | 18 | 90 | 28 |
| ,, | 200 | 24 | 200 | 42 |
| MeCN | 30 | 46 | 25 | 66 |
| ,, | 50 | 57 | 50 | 99 |
| ,, | 90 | 93 | 200 | 99 |
| ,, | — | — | 20 hr. | 99 |

*Formation and Assay of Tetrabenzyl Pyrophosphate from* cyclo*Pentanone Oxime* p-*Nitrobenzenesulphonate.*—In a typical experiment a solution of dibenzyl hydrogen phosphate (0·556 g., 0·002 mole) and 2 : 6-lutidine (0·11 c.c., 0·001 mole) was prepared in nitromethane (5 c.c.), *cyclo*pentanone oxime *p*-nitrobenzenesulphonate (0·284 g., 0·001 mole) added, the whole kept at room temperature for 16 hr. and then evaporated under reduced pressure. The residue was dissolved in chloroform (40 c.c.), the solution washed with water (20 c.c.), saturated sodium hydrogen carbonate solution (20 c.c.), and water (20 c.c.), then dried ($Na_2SO_4$) and evaporated. The neutral residue was assayed for tetrabenzyl pyrophosphate in the following way. The residue was dissolved in benzene (10 c.c.), and *cyclo*hexylamine (0·5 c.c., 0·005 mole) added; precipitation began almost immediately. The mixture was set aside overnight, then filtered, and the solid washed with benzene (5 c.c.) and ether (5 c.c.) and dried at 60° *in vacuo* over phosphoric oxide. The product, *cyclo*hexylammonium dibenzyl phosphate (0·299 g., 80%), had m. p. 170—172°. Crystallisation from ethanol gave the pure salt (0·21 g.), m. p. and mixed m. p. 173°. The filtrate and washings from the *cyclo*hexylamine fission were combined and successively washed with 0·1N-sodium hydroxide (5 c.c.), water (5 c.c.), 0·1N-hydrochloric acid (5 c.c.), and water (5 c.c.), dried ($Na_2SO_4$), and evaporated under reduced pressure. The residue crystallised from *cyclo*hexane, giving dibenzyl *N-cyclo*hexylphosphoramidate (0·14 g., 39%), m. p. and mixed m. p. 78—80°.

In this and subsequent experiments involving the *cyclo*hexylamine assay the yield of tetrabenzyl pyrophosphate was based on the amount of *cyclo*hexylammonium dibenzyl phosphate isolated from the neutral fraction.

The above experiment was repeated with different solvents and varying proportions of base: the results are tabulated.

| Solvent | Reaction time (hr.) | Base | Yield of pyrophosphate (%) |
|---|---|---|---|
| Nitromethane | 16 | Triethylamine (1 mol.) | 80 |
| ,, | ,, | ,, (3 mol.) | 75 |
| ,, | ,, | 2 : 6-Lutidine (1 mol.) | 80 |
| ,, | ,, | ,, (3 mol.) | 35 |
| Pyridine | 20 | None ,, | 0 |

*Tetrabenzyl Pyrophosphate from* cyclo*Pentanone Oxime* p-*Nitrobenzenesulphonate and Dibenzyl Hydrogen Phosphate.*—To a solution of dibenzyl hydrogen phosphate (11·12 g., 0·04 mole) and triethylamine (2·6 c.c., 0·02 mole) in dry nitromethane (50 c.c.), *cyclo*pentanone oxime *p*-nitrobenzenesulphonate (5·68 g., 0·2 mole) was added. The solution warmed spontaneously from 17° to *ca.* 25° and was kept overnight at room temperature. The solution was then diluted with chloroform (100 c.c.) and washed with saturated sodium hydrogen carbonate solution (100 c.c.) and with water (2 × 100 c.c.), then dried ($Na_2SO_4$) and evaporated under reduced pressure. The residue was dissolved in acetone (10 c.c.) and poured into water. The gummy precipitate was collected and dried at room temperature *in vacuo* over phosphoric oxide. Recrystallisation from benzene–light petroleum (b. p. 60—80°) afforded tetrabenzyl pyrophosphate (9·22 g., 86%), m. p. and mixed m. p. 59—61°.

*Beckmann Rearrangement of* cyclo*Pentanone Oxime* p-*Nitrobenzenesulphonate followed by Reaction with Dibenzyl Hydrogen Phosphate.*—A solution of *cyclo*pentanone oxime *p*-nitrobenzenesulphonate (0·284 g., 0·001 mole) in nitromethane (5 c.c.) was kept at room temperature for 2 hr.; rearrangement to the *O-p*-nitrobenzenesulphonate of valerolactam was then complete. Triethylamine (0·13 c.c., 0·001 mole) and dibenzyl hydrogen phosphate (0·556 g., 0·002 mole) were added, and the solution was set aside at room temperature for 16 hr. The neutral fraction was isolated as described above and assayed with *cyclo*hexylamine. The yield of tetrabenzyl

pyrophosphate was 27%. In a similar experiment where the amount of triethylamine added after the Beckmann rearrangement was increased to 0·003 mole the yield of pyrophosphate was increased to 44%.

*Preparation of Tetrabenzyl Pyrophosphate from* cyclo*Pentanone Oxime Benzenesulphonate.*—The reactions involving the formation of tetrabenzyl pyrophosphate and its subsequent assay by treatment with *cyclo*hexylamine and isolation of *cyclo*hexylammonium dibenzyl phosphate were repeated as described above, but with *cyclo*pentanone oxime benzenesulphonate in place of *p*-nitrobenzenesulphonate, and 2 : 6-lutidine (1 mol.) in place of triethylamine. When 1 mol. of oxime ester was employed, the yield of tetrabenzyl pyrophosphate was 41% in methyl cyanide and 55% in nitromethane; with nitromethane as solvent but with 2 mols. of oxime ester yields of 75—80% were obtained.

*Tetrabenzyl Pyrophosphate from Acetophenone Oxime Toluene-*p*-sulphonate.*—From acetophenone oxime toluene-*p*-sulphonate (0·289 g., 0·001 mole), dibenzyl hydrogen phosphate (0·55 g., 0·002 mole), and triethylamine (0·13 c.c., 0·001 mole) in benzene (5 c.c.) at 18° for 16 hr. the yield of tetrabenzyl pyrophosphate was 16%. The use of methyl cyanide (5 c.c.) increased the yield to 22%.

*Tetrabenzyl Pyrophosphate from Ethyl Methyl Ketoxime Benzenesulphonate.*—In a typical experiment ethyl methyl ketoxime benzenesulphonate (0·227 g., 0·001 mole) was added to a solution of dibenzyl hydrogen phosphate (0·556 g., 0·002 mole) and triethylamine (0·13 c.c., 0·001 mole) in methyl cyanide (10 c.c.), the solution kept at 18° for 16 hr., and the isolated neutral fraction assayed for tetrabenzyl pyrophosphate with *cyclo*hexylamine in the usual manner. The results are tabulated for 0·001 mole of base in 10 c.c. of solvent.

| Solvent | Base added | Temp. | Reaction time (hr.) | Yield of pyrophosphate (%) |
|---|---|---|---|---|
| $MeNO_2$ ..... | Triethylamine | 18° | 16 | 38 |
| " ..... | 2 : 6-Lutidine | 18 | 60 | 34 |
| " ..... | " | 80 | 2 | 35 |
| $C_6H_6$ ..... | " | 18 | 16 | 20 |

*Preparation of Dibenzyl Diphenyl Pyrophosphate from* cyclo*Pentanone Oxime* p*-Nitrobenzenesulphonate.*—An aqueous solution of diphenyl hydrogen phosphate (0·25 g., 0·001 mole) was titrated to pH 7 with tetramethylammonium hydroxide solution, the solution then evaporated under reduced pressure and dried by repeated evaporation with benzene. The residue was dissolved in benzene (10 c.c.), *cyclo*pentanone oxime *p*-nitrobenzenesulphonate (0·284 g., 0·001 mole) added, and the solution refluxed for 45 min. during which an oil was precipitated which solidified on cooling. The supernatant solution was decanted from the solid which was extracted with benzene (3 c.c.). The combined benzene solutions were refluxed for 2 hr. with dibenzyl hydrogen phosphate (0·278 g., 0·001 mole), cooled, washed with water (5 c.c.), and dried ($Na_2SO_4$). The solution was treated with *cyclo*hexylamine (0·5 c.c., 0·004 mole) and kept at room temperature for 16 hr.; then the precipitate formed (0·235 g., 71%), m. p. 172—179°, was filtered off. After crystallisation from ethanol the precipitate gave *cyclo*hexylammonium diphenyl phosphate (0·2 g.), m. p. and mixed m. p. 190°. The benzene filtrate from the *cyclo*hexylamine treatment was washed with N-hydrochloric acid (3 × 5 c.c.), water (5 c.c.), N-sodium hydroxide (3 × 5 c.c.), and water (5 c.c.). The dried ($Na_2SO_4$) solution was evaporated, and the residue dissolved in ethanol (2 c.c.) and poured into water (45 c.c.). The precipitate (0·172 g.), m. p. 70—73°, after crystallisation from *cyclo*hexane gave dibenzyl *N-cyclo*hexylphosphoramidate (0·11 g.), m. p. and mixed m. p. 78—80°.

Use of methyl cyanide instead of benzene in the above reaction of tetraethylammonium diphenyl phosphate with the oxime sulphonate gave a mixture of pyrophosphate tetraesters as judged by the *cyclo*hexylamine fission of the isolated neutral fraction. Similarly, treatment of tetraethylammonium dibenzyl phosphate with the oxime sulphonate in nitromethane followed by reaction with diphenyl hydrogen phosphate gave mixtures of pyrophosphate derivatives.

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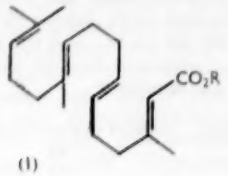
UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, September 21st,* 1955.]

## 256. *Reduced Cyclic Compounds. Part I. The Preparation and Cyclodehydration of ω-Phenyl Unsaturated Tertiary Alcohols.*

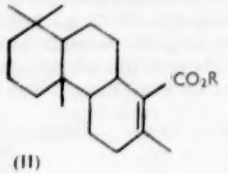
By M. F. Ansell and M. E. Selleck.

The preparations are reported of 2-methyl-9-phenylnon-6-en-2-ol and 2-methyl-13-phenyltrideca-6 : 10-dien-2-ol. These have been converted into 1 : 2 : 3 : 4 : 4*a* : 9 : 10 : 10*a*-octahydro-1 : 1-dimethylphenanthrene and 1 : 2 : 3 : 4 : 4*a* : 4*b* : 5 : 6 : 10*b* : 11 : 12 : 12*a*-dodecahydro-1 : 1-dimethylchrysene respectively.

The formation of reduced cyclic systems from long-chain unsaturated compounds has been suggested as a possible biogenetic route to terpenes and steroids.[1,2] That this type of reaction is possible has been shown by Caliezi and Schinz[3] who converted the unsaturated open-chain ester (I) into the tricyclic ester (II) by treatment with formic acid containing a small amount of sulphuric acid.




(I)

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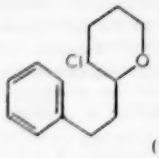


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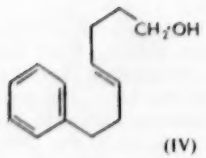


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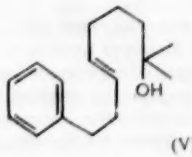


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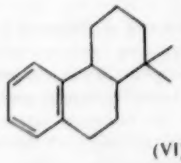

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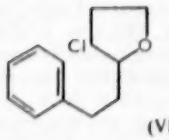


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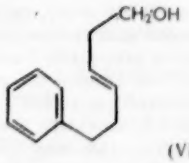
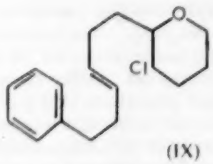



(VIII)

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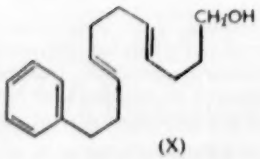
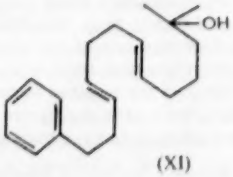
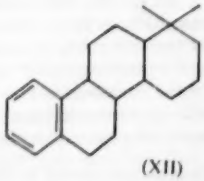



(X)




(XI)



(XII)

The object of the present work was the preparation of completely synthetic unsaturated alcohols capable of undergoing cyclodehydration. For this purpose the method of chain-extension by means of 1 : 2-dichlorotetrahydro-furans or -pyrans, developed by Crombie

[1] Woodward and Bloch, *J. Amer. Chem. Soc.*, 1953, **75**, 2023.
[2] Ruzicka, *Experientia*, 1953, **9**, 357.
[3] Caliezi and Schinz, *Helv. Chim. Acta*, 1952, **35**, 1649.

and Harper,[4] is satisfactory: a double bond may be introduced at a known position, and a terminal hydroxyl group is available for further chain-extension.

The Grignard reagent from phenethyl bromide, with 2 : 3-dichlorotetrahydropyran, gave smoothly 3-chlorotetrahydro-2-phenethylpyran (III), which on ring scission with sodium gave 7-phenylhept-4-en-1-ol (IV). Conversion of the latter into 7-phenylheptane-1 : 3 : 4-triol followed by oxidation with periodic acid gave 3-phenylpropanol. The other product of the oxidation, 4-hydroxybutanal, could not be isolated. Previous work[5] has shown this to be expected. These results confirm expected position for the double bond. Conversion of the alcohol (IV) into 2-methyl-9-phenylnon-6-en-2-ol (V) was accomplished by reaction of acetone with the Grignard reagent derived from the corresponding bromide. This compound is the *trans*-isomer as the double bond was introduced by opening a 3-chlorotetrahydropyran ring.[4a]

Treatment of the tertiary alcohol (V) with cold concentrated sulphuric acid or hot polyphosphoric acid gave 1 : 2 : 3 : 4 : 4*a* : 9 : 10 : 10*a*-octahydro-1 : 1-dimethylphenanthrene (VI). The structure of the product, which contained no ethylenic double bonds, was established by oxidation to phthalic acid, and dehydrogenation to 1-methylphenanthrene. In addition its ultraviolet spectrum had absorption maxima at 267 and 273 mμ, which are identical with those found by Askew[6] for 1 : 2 : 3 : 4 : 4*a* : 9 : 10 : 10*a*-octahydrophenanthrene.

This synthetic route was extended to the preparation of 2-methyl-13-phenyltrideca-6 : 10-dien-2-ol (XI). 3-Chlorotetrahydro-2-phenethylfuran (VII), obtained from phenethylmagnesium bromide and 2 : 3-dichlorotetrahydrofuran, on treatment with sodium gave 6-phenylhex-3-en-1-ol (VIII). Application of the chain-extension procedure then gave the chloride (IX) and thence 11-phenylundeca-4 : 8-dien-1-ol (X), which was converted in two stages into the required tertiary alcohol (XI). This product was not stereochemically pure, being, from its method of preparation, a mixture of 6 : 7-*trans*-10 : 11-*cis*- and 6 : 7-*trans*-10 : 11-*trans*-forms, the latter probably predominating.

Cyclodehydration of this substituted tridecadienol (XI) with cold sulphuric acid gave 1 : 2 : 3 : 4 : 4*a* : 4*b* : 5 : 6 : 10*b* : 11 : 12 : 12*a*-dodecahydro-1 : 1-dimethylchrysene (XII). This structure is based on the saturated nature of the product, its dehydrogenation (and demethylation) to 1-methylchrysene, and its ultraviolet absorption (max. 267 and 273 mμ) which is similar to that of 1 : 2 : 3 : 4 : 4*a* : 9 : 10 : 10*a*-octahydrophenanthrene.

The above synthetic route is applicable to the preparation of completely aliphatic compounds, and, by the use of suitable methyl-substituted dihydropyrans and dihydrofurans, to the preparation of unsaturated alcohols which on cyclodehydration will yield cyclic systems containing angular methyl groups. Both these aspects are under investigation.

## Experimental

*7-Phenylhept-3-en-1-ol.*—A solution of 2 : 3-dichlorotetrahydropyran[4a] (from 2 : 3-dihydropyran, 45 g.) in ether (150 c.c.) was added dropwise to a stirred (Hershberg wire stirrer) solution of the Grignard reagent prepared from phenethyl bromide[7] (139 g.) and magnesium (18·5 g.) in ether (350 c.c.) at such a rate that steady refluxing was maintained. Before all the 2 : 3-dichlorotetrahydropyran had been added the reaction mixture set solid, addition was stopped, and the mixture left overnight. Excess of Grignard reagent was decomposed with ammonium chloride solution, followed by sufficient concentrated hydrochloric acid to give a clear solution. The ethereal layer was separated and the aqueous layer extracted with ether. The ether was removed from the dried ($K_2CO_3$) combined ether solutions and the residue distilled, giving, after elimination of lower-boiling material, a mixture of cis- and trans-3-*chlorotetrahydro-2-phenethylpyran* (65 g.), b. p. 74—110°/0·006 mm. (Found: C, 70·3; H, 7·7; Cl, 15·3. $C_{13}H_{17}OCl$ requires C, 69·5; H, 7·6; Cl, 15·8%).

[4] Crombie and Harper, *J.*, 1950, (*a*) 1707, (*b*) 1714.
[5] Paul, *Compt. rend.*, 1941, **212**, 492.
[6] Askew, *J.*, 1935, 512.
[7] Slotta and Altner, *Ber.*, 1931, **64**, 1510.

*7-Phenylhept-4-en-1-ol.*—Sodium (30 g.) was powdered under xylene, then thoroughly rinsed with dry ether and covered with this solvent (250 c.c.). A little 3-chlorotetrahydro-2-phenethylpyran was added and the suspension stirred until reaction commenced, as shown by the surface of the sodium assuming an indigo-blue colour and by refluxing of the ether. The remaining 3-chlorotetrahydro-2-phenethylpyran (106 g. in all) was then added dropwise so that steady refluxing was maintained. The thick blue suspension was stirred for a further 2 hr. and then kept at room temperature overnight. Next day ice and water were cautiously added. The ether layer was separated and the aqueous layer extracted with ether. After being washed and dried ($Na_2SO_4$), the ether was removed from the combined extracts, and the residue was distilled, to give *7-phenylhept-4-en-1-ol* (74 g.), b. p. 110—118°/0·7 mm., $n_D^{20}$ 1·5237 (Found : C, 82·0; H, 9·7. $C_{13}H_{18}O$ requires C, 82·1; H, 9·5%). The *3 : 5-dinitrobenzoate* (from light petroleum, b. p. <40°) had m. p. 42·5—43·5° (Found : C, 62·5; H, 5·7; N, 7·15. $C_{20}H_{20}O_6N_2$ requires C, 62·5; H, 5·2; N, 7·3%).

*7-Phenylheptane-1 : 3 : 4-triol.*—7-Phenylhept-4-en-1-ol (5 g.) was added to a stirred mixture of 100% formic acid (50 c.c.) and 30% hydrogen peroxide (3·5 g.). The temperature rose to 46° and was kept, by heating, at 40° for 2 hr. After cooling, the mixture was diluted with water, and the oil which separated extracted with ether. The ethereal extract was evaporated and the residue boiled with excess of 10% sodium hydroxide solution for 45 min., cooled, neutralised with hydrochloric acid, and extracted with ether. After drying ($MgSO_4$), the ethereal extract was evaporated, to give *7-phenylheptane-1 : 3 : 4-triol* (0·5 g.), m. p. 82—83° after recrystallisation from benzene–light petroleum (b. p. 60—80°) (Found : C, 68·7; H, 9·1. $C_{13}H_{20}O_3$ requires C, 69·6; H, 8·9%).

*Periodic acid fission.* The triol (0·4 g. in methyl alcohol (5 c.c.) was shaken for 12 hr. with periodic acid (0·5 g.), then diluted with water, and extracted with ether. The residue obtained on evaporation of the ethereal solution was boiled with an aqueous-alcoholic sulphuric acid solution of 2 : 4-dinitrophenylhydrazine, yielding 3-phenylpropanal 2 : 4-dinitrophenylhydrazone as orange plates, m. p. and mixed m. p. 158° after recrystallisation from benzene–light petroleum (b. p. 60—80°) (Found : C, 57·3; H, 4·6; N, 17·9. Calc. for $C_{15}H_{14}O_4N_4$ : C, 57·3; H, 4·5; N, 17·8%). Triebs [8] records m. p. 146—147°.

*7-Bromo-1-phenylhept-3-ene.*—A mixture of 7-phenylhept-4-en-1-ol (45 g.) and pyridine (6 c.c.) was added slowly with stirring to phosphorus tribromide (26 g.), with ice-cooling; after being kept overnight at room temperature the products were distilled (b. p. 110—140°/0·7 mm.) and the distillate shaken with water and then with 10% sodium carbonate solution. The organic layer, dried ($CaCl_2$) and distilled, yielded *7-bromo-1-phenylhept-3-ene* (30 g.), b. p. 108—110°/0·37 mm., $n_D^{20}$ 1·5382 (Found : C, 62·3; H, 6·9; Br, 31·6. $C_{13}H_{17}Br$ requires C, 61·7; H, 6·7; Br, 31·6%). The derived *thiuronium picrate* formed yellow plates (from alcohol), m. p. 162—163° (Found : C, 50·3; H, 4·8; N, 14·8. $C_{20}H_{23}O_7N_5S$ requires C, 50·3; H, 5·1; N, 15·3%).

*2-Methyl-9-phenylnon-6-en-2-ol.*—Acetone (7 g.) was added to a stirred solution of the Grignard reagent from 7-bromo-1-phenylhept-3-ene (28 g.) and magnesium (3 g.) in ether (60 c.c.) at such a rate that steady refluxing was maintained. Next morning excess of 10% sulphuric acid was added, the ethereal layer separated, and the aqueous layer extracted with ether. Removal of the ether from the dried ($K_2CO_3$) combined extracts followed by distillation of the residue gave *2-methyl-9-phenylnon-6-en-2-ol* (21 g.), b. p. 110—114°/2 × $10^{-3}$ mm., $n_D^{20}$ 1·5105 (Found : C, 82·3; H, 10·4. $C_{16}H_{24}O$ requires C, 82·8; H, 10·3%).

*Cyclodehydration.* (*a*) 2-Methyl-9-phenylnon-6-en-2-ol (4 g.) was added dropwise during 40 min. with stirring to ice-cold 90% sulphuric acid (10 c.c.). The ice-bath was removed and stirring continued for a further 2 hr. The mixture was diluted with water to 50 c.c. and extracted with ether. After being washed with water and 10% sodium carbonate solution and dried ($CaCl_2$), the ethereal extract was evaporated and the residue distilled over sodium, yielding *1 : 2 : 3 : 4 : 4a : 9 : 10 : 10a-octahydro-1 : 1-dimethylphenanthrene* (1 g.), b. p. 170°/10 mm., $n_D^{20}$ 1·5454—1·5470 (Found : C, 89·9; H, 10·25. $C_{16}H_{22}$ requires C, 89·7; H, 10·3%). (*b*) The alcohol (10 g.) was heated with phosphoric oxide (10 g.) in 85% phosphoric acid (50 c.c.) at 160° for 3 hr. with stirring. The mixture was diluted with water and extracted with ether. The ethereal extract was worked up as in (*a*), yielding *1 : 2 : 3 : 4 : 4a : 9 : 10 : 10a-octahydro-1 : 1-dimethylphenanthrene* (3·8 g.), b. p. 104—109/0·7 mm., $n_D^{20}$ 1·5464—1·5476. This compound was saturated towards hydrogen at atmospheric pressure in the presence of Adams catalyst.

[8] Triebs, *Ber.*, 1947, **80**, 97.

*Dehydrogenation of* 1 : 2 : 3 : 4 : 4a : 9 : 10 : 10a-*Octahydro*-1 : 1-*dimethylphenanthrene*.—The above hydrocarbon (1 g.) was heated with selenium powder (1·5 g.) for 5 hr. at 300—330°, then extracted with alcohol, and the extract was boiled with charcoal and filtered. On concentration the filtrate deposted 1-methylphenanthrene [9] (0·3 g.), m. p. and mixed m. p. 118—119° after recrystallisation from light petroleum (b. p. 40—60°) (picrate, m. p. 135—136°).

3-*Chlorotetrahydro*-2-*phenethylfuran*.—2 : 3-Dichlorotetrahydrofuran [4] (140 g., 1 mole) was added to a stirred solution of the Grignard reagent prepared from phenethyl bromide (277 g., 1·5 mole) and magnesium (36 g., 1·5 g.-atom) in ether (1 l.) at such a rate that steady refluxing was maintained. The mixture was left overnight and then worked up as for 3-chlorotetrahydro-2-phenethylpyran. Distillation, after elimination of lower-boiling material (ethylbenzene), gave a mixture of *cis*- and *trans*-3-chlorotetrahydro-2-phenethylfuran (164 g., 78%) b. p. 70—100°/$10^{-2}$ mm., $n_D^{20}$ 1·5319—1·5340 (Found : C, 69·8; H, 7·6; Cl, 16·0. Calc. for $C_{12}H_{15}OCl$ : C, 68·4; H, 7·1; Cl, 16·8%).

6-*Phenylhex*-3-*en*-1-*ol*.—Ring scission of 3-chlorotetrahydro-2-phenethylfuran (290 g.) was effected as for the pyran, with sodium (70 g.) in ether (1 l.). The final distillation gave a mixture of *cis*- and *trans*-6-phenylhex-3-en-1-ol (216 g., 88·9%), b. p. 100—110°/0·7 mm., $n_D^{20}$ 1·5289 (Found : C, 82·15; H, 8·8. Calc. for $C_{12}H_{16}O$ : C, 81·8; H, 9·1%) [3 : 5-dinitrobenzoate, m. p. 63—64°, from light petroleum (b. p. 40°) (Found : C, 61·6; H, 5·3; N, 7·5. Calc. for $C_{19}H_{18}O_6N_2$ : C, 61·6; H, 4·9; N, 7·6%)].

1-*Bromo*-6-*phenylhex*-3-*ene*.—A mixture of 6-phenylhex-3-en-1-ol (216 g.) and pyridine (27 c.c.) was added dropwise with stirring to phosphorus tribromide (130 g.), with ice-cooling. The subsequent procedure was as for 7-bromo-1-phenylhept-3-ene. The final distillation gave 1-*bromo*-6-*phenylhex*-3-*ene* (143 g.), b. p. 55—92°/8 × $10^{-3}$ mm., $n_D^{20}$ 1·5446 (Found : C, 60·5; H, 6·4; Br, 32·9. $C_{12}H_{15}Br$ require C, 60·25; H, 6·3; Br, 33·5%) [*thiouronium picrate*, yellow needles (from alcohol), m. p. 164—165° (Found : C, 49·6; H, 4·5. $C_{19}H_{21}O_7N_6S$ requires C, 49·2; H, 4·5%)].

11-*Phenylundeca*-4 : 8-*dien*-1-*ol*.—The Grignard reagent from 1-bromo-6-phenylhex-3-ene (143 g.) and magnesium (16 g.) in ether (250 c.c.) was left overnight, and the clear solution then decanted and treated (stirring) with 2 : 3-dichlorotetrahydropyran (34 g.) in ether (100 c.c.) at such a rate that steady refluxing was maintained. Next morning the mixture was worked up as for the pyran. Distillation gave 6-phenylhex-3-ene (28 g.), b. p. 102/15 mm., $n_D^{17.5}$ 1·5050 (Found : C, 89·6; H, 10·5. Calc. for $C_{12}H_{16}$ : C, 90·0; H, 10·0%) (Triebs [9] gives b. p. 222°, $n_D^{20}$ 1·5039), and 3-chlorotetrahydro-2-(6-phenylhex-3-enyl)pyran (65 g.) b. p. 114—140°/3 × $10^{-3}$ mm. This crude material was subjected to ring scission as for the pyran, with sodium (13 g.) in ether (300 c.c.). Distillation gave 11-*phenylundeca*-4 : 8-*dien*-1-*ol* (44 g.), b. p. 120—138°/3 × $10^{-3}$ mm., $n_D^{20}$ 1·5219 (Found : C, 83·5; H, 10·1. $C_{17}H_{24}O$ requires C, 83·6; H, 9·8%).

11-*Bromo*-1-*phenylundeca*-3 : 7-*diene*.—A mixture of the preceding alcohol (40 g.) and pyridine (4 c.c.) was added with stirring to phosphorus tribromide (20 g.), with ice-cooling. The subsequent procedure was as for 7-bromo-1-phenylhept-3-ene. Distillation gave 11-*bromo*-1-*phenylundeca*-3 : 7-*diene* (25 g.), b. p. 140—145°/6 × $10^{-4}$ mm., $n_D^{20}$ 1·5325 (Found : C, 67·0; H, 7·7. $C_{17}H_{23}Br$ requires C, 66·45; H, 7·5%). The derived *thiuronium picrate* formed yellow needles (from alcohol), m. p. 162—163° (Found : C, 54·1; H, 5·8. $C_{24}H_{29}O_7N_6S$ requires C, 54·2; H, 5·5%).

2-*Methyl*-13-*phenyltrideca*-6 : 10-*dien*-2-*ol*.—Acetone (4·5 g.) in ether (10 c.c.) was added to a solution of the Grignard reagent from 11-bromo-1-phenylundeca-3 : 7-diene (22 g.) and magnesium (2·5 g.) in ether (10 c.c.) at such a rate that steady refluxing was maintained. The subsequent procedure was as for 2-methyl-9-phenylnon-6-en-2-ol. Distillation gave 2-*methyl*-13-*phenyltrideca*-6 : 10-*dien*-2-*ol* (10 g.), b. p. 130—145°/2 × $10^{-3}$ mm., $n_D^{20}$ 1·5117 (Found : C, 84·15; H, 10·65. $C_{20}H_{30}O$ requires C, 83·9; H, 10·5%).

*Cyclodehydration*. The alcohol (7·5 g.) was treated with 90% sulphuric acid (15 c.c.) as for 2-methyl-9-phenylnon-6-en-2-ol. The mixture was extracted with light petroleum (b. p. 40—60°), and the extract washed with concentrated sulphuric acid. After being kept over potassium carbonate the extract was filtered and the light petroleum removed by distillation. The residue was distilled over sodium, yielding 1 : 2 : 3 : 4 : 4a : 4b : 5 : 6 : 10b : 11 : 12 : 12a-*dodecahydro*-1 : 1-*dimethylchrysene* (3 g.), b. p. 120—130°/3 × $10^{-3}$ mm., $n_D^{20}$ 1·5520 (Found : C, 89·45; H, 10·7. $C_{20}H_{28}$ requires C, 89·55; H, 10·45%), saturated towards hydrogen at atmospheric pressure in the presence of Adams catalyst.

[9] Haworth, *J.*, 1932, 1125.

*Dehydrogenation of* 1 : 2 : 3 : 4 : 4a : 4b : 5 : 6 : 10b : 11 : 12 : 12a-*Dodecahydro*-1 : 1-*dimethylchrysene*.—The above hydrocarbon (1 g.) was heated with selenium powder (2·5 g.) at 350—400° for 5 hr. After cooling, the mixture was extracted with alcohol, and the extract boiled with charcoal and then filtered. On cooling, crystals of 1-methylchrysene were deposited which, crystallised from alcohol and then hexane, sublimed under reduced pressure and, recrystallised from hexane, had m. p. 247—249°. Ruzicka [10] gives m. p. 254—255° (*in vacuo*).

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MILE END ROAD, LONDON, E.1. [*Received, October 6th*, 1955.]

[10] Ruzicka, *Helv. Chim. Acta*, 1940, **23**, 388.

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## 257. *Complex Fluorides. Part V.* Complexes formed by Molybdenum Hexafluoride, Tungsten Hexafluoride, and Rhodium Tetrafluoride.*

By B. COX, D. W. A. SHARP, and A. G. SHARPE.

The hexafluorides of molybdenum and tungsten combine with potassium, rubidium, and cæsium fluorides to form complexes of general formulæ $M_2MoF_8$ and $M_2WF_8$. The complex fluoride which is obtained by interaction of bromine trifluoride and sodium hexachlororhodate(III) is sodium hexafluororhodate(IV), $Na_2RhF_6$.

RUFF and EISNER [1,2] reported that the hexafluorides of molybdenum and tungsten combine with alkali-metal fluorides, but gave no details and described no compounds. The preparation of complexes of uranium hexafluoride [3,4] (*e.g.*, $Na_3UF_9$, $K_2UF_8$) suggested to us the possibility of finding evidence for unusual co-ordination numbers in complexes derived from molybdenum and tungsten hexafluorides, and we have therefore re-examined the interaction of these substances and the fluorides of sodium, potassium, rubidium, and cæsium.

Molybdenum hexafluoride has usually been made by the action of fluorine on the powdered metal; for the present purpose, however, the use of bromine trifluoride as the fluorinating agent has proved more convenient. The hexafluoride obtained by this method is contaminated with bromine, but for chemical work this is not a serious disadvantage, and the possibility of carrying out the preparation in a closed system greatly simplifies the essential task of rigidly excluding moisture. After any excess of bromine trifluoride had been frozen out, the mixture of molybdenum hexafluoride and bromine was condensed on the alkali-metal fluoride, absorption of the hexafluoride was allowed to take place, then the excess of molybdenum hexafluoride and the whole of the bromine were removed by distillation. The products obtained from potassium, rubidium, and cæsium fluorides had the general formula $M_2MoF_8$, and on a second treatment with the hexafluoride underwent no change in weight. Sodium fluoride absorbed only a negligible amount of the hexafluoride.

Essentially the same procedure was applied in the preparation of complexes of tungsten hexafluoride, and the compounds $K_2WF_8$, $Rb_2WF_8$, and $Cs_2WF_8$ were obtained; sodium fluoride again failed to react. All of the complex fluorides prepared are stable at room temperature in the absence of moisture; we have so far, however, been unable to obtain

* Part IV, *J.*, 1956, 876.

[1] Ruff and Eisner, *Ber.*, 1905, **38**, 742.
[2] *Idem*, *Ber.*, 1907, **40**, 2926.
[3] Martin and Albers, *Naturwiss.*, 1946, **33**, 370.
[4] Martin, Albers, and Dust, *Z. anorg. Chem.*, 1951, **265**, 128.

satisfactory *X*-ray powder photographs of them or to learn anything concerning their structures. All are decomposed by bromine trifluoride, yielding the tetrafluorobromites.

It has been shown [5] that the action of bromine trifluoride on sodium hexachlororhodate(III), or on mixtures of sodium and rhodium halides, produces impure complex fluorides containing tetrapositive rhodium; the oxidation state of the rhodium has been confirmed by magnetic measurements.[6] It was originally thought that the pale blue product obtained from sodium hexachlororhodate had the formula $Na_3RhF_7$, and was analogous to the complex $K_3CoF_7$ described by Klemm and Huss.[7] Subsequent investigations by Klemm and his co-workers [8,9] have shown that the compound to which the formula $K_3CoF_7$ was assigned is really $K_3CoF_6$, and that yellow complexes of formulæ $K_2RhF_6$, $Rb_2RhF_6$, and $Cs_2RhF_6$ are obtained by the action of fluorine on mixtures of alkali-metal and rhodium halides at about 320°. The interaction of bromine trifluoride and sodium hexachlororhodate(III) has therefore been re-investigated, making use of a technique first employed by Zachariasen [10]—the identification of a substance by its predicted *X*-ray powder pattern. From the lattice constants [11] of the isomorphous sodium salts of general formula $Na_2XF_6$, and the similarity in lattice constants [9,12] of $Cs_2RhF_6$ and $Cs_2PtF_6$, it would be expected that $Na_2RhF_6$ and $Na_2PtF_6$ would be isomorphous and almost isodimensional. The fact that the principal phase present in the products obtained from bromine trifluoride and sodium chlororhodate or mixtures of sodium and rhodium chlorides is isomorphous and nearly isodimensional with $Na_2PtF_6$ and $Na_2PdF_6$ provides strong evidence for the formation of the complex $Na_2RhF_6$ in these reactions; the blue colour is perhaps due to the presence of a little rhodium tetrafluoride [6] arising by solvolysis of the complex. Lattice constants for $Na_2RhF_6$, which has a hexagonal unit cell, are $a = 9{\cdot}32$, $c = 5{\cdot}22$ Å; those for the isomorphous sodium hexafluoropalladate, which has been prepared for the first time during this investigation, are $a = 9{\cdot}23$, $c = 5{\cdot}25$ Å. The formation of the hexafluororhodate(IV) was further substantiated by showing that interaction of bromine trifluoride and mixtures of cæsium and rhodium chlorides gave only cæsium hexafluororhodate(IV), $Cs_2RhF_6$, identified by analysis and by comparison of its *X*-ray powder diagram with the data given by Weise and Klemm.[9]

## Experimental

Reactions involving bromine trifluoride and *X*-ray powder photographic examinations were carried out as described in previous papers. For the study of complex formation by molybdenum and tungsten hexafluorides apparatus essentially similar to that described by Emeléus and Woolf [13] was employed. All operations on complex fluorides of molybdenum, tungsten, and rhodium were conducted in rigorously dried, closed systems or in a " dry-box."

*Complexes of Molybdenum Hexafluoride.*—Molybdenum powder was allowed to react with bromide trifluoride, and the resulting volatile products were passed through a trap at −23° (to remove excess of bromine trifluoride) and condensed at −196°. The mixture of molybdenum hexafluoride and bromine was then distilled on to a weighed quantity of the alkali-metal fluoride contained in a quartz flask, and, absorption of the hexafluoride having occurred, the excess of the latter compound and the whole of the bromine were removed by distillation. Alkali metal in the white residue was determined from the weight of halide taken or as chloroplatinate (after removal of molybdenum by treatment with bromine trifluoride, and decomposition of the resulting tetrafluorobromite by exposure to moist air); molybdenum was precipitated as its 8-hydroxyquinoline complex (with the formation of which fluoride ion was shown in model determinations not to interfere); fluorine was determined gravimetrically as calcium fluoride (Found, for the K *salt*: K, 24·1; Mo, 28·8. $K_2MoF_8$ requires K, 23·9; Mo, 30·1. Found, for

[5] Sharpe, *J.*, 1950, 3444.
[6] Nyholm and Sharpe, *J.*, 1952, 3579.
[7] Klemm and Huss, *Z. anorg. Chem.*, 1949, **258**, 221.
[8] Klemm, *Angew. Chem.*, 1954, **66**, 468.
[9] Weise and Klemm, *Z. anorg. Chem.*, 1953, **272**, 211.
[10] Zachariasen, *Acta Cryst.*, 1948, **1**, 265; *J. Amer. Chem. Soc.*, 1948, **70**, 2147.
[11] Cox, *J.*, 1954, 3251.
[12] Cox and Sharpe, *J.*, 1953, 1783.
[13] Emeléus and Woolf, *J.*, 1950, 164.

the Rb *salt*: Rb, 44·0; Mo, 21·7; F, 34·5. $Rb_2MoF_8$ requires Rb, 40·7; Mo, 22·9; F, 36·2. Found, for the Cs *salt*: Cs, 48·7; Mo, 19·7; F, 31·6. $Cs_2MoF_8$ requires Cs, 51·7; Mo, 18·7; F, 29·6%).

*Complexes of Tungsten Hexafluoride.*—The experimental methods were analogous to those described in the preceding section. Tungsten was determined by the cinchonine hydrochloride–tannin method and ignition to the trioxide (Found, for the K *salt*: W, 44·9, 42·0. $K_2WF_8$ requires W, 44·4. Found, for the Rb *salt*: Rb, 33·9; W, 35·6. $Rb_2WF_8$ requires Rb, 33·7; W, 36·3. Found, for the Cs *salt*: Cs, 45·3; W, 28·9. $Cs_2WF_8$ requires Cs, 44·2; W, 30·5%).

*Complexes of Rhodium Tetrafluoride.*—Preparations and analyses for sodium compounds were as described previously.[5] Cæsium hexafluororhodate(IV) was made by interaction of bromine trifluoride and a mixture of cæsium chloride and hydrated rhodium chloride containing cæsium and rhodium in 2 : 1 proportions; the product was heated at 270° *in vacuo* (Found: F, 24·4. Calc. for $Cs_2RhF_8$: F, 23·6%). The powder pattern of the product was indexed on the basis of a hexagonal unit cell with $a = 6{\cdot}28$, $c = 10{\cdot}11$ Å, in agreement with values previously reported for the high-temperature form of this substance by Weise and Klemm.[9] *X*-Ray powder photography revealed no evidence for the formation of other complex fluorides of cæsium (or sodium) and rhodium when the proportions of the starting materials were varied.

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## NOTE.

### 258. *The Action of Phosphorus Pentachloride on* 1 : 2-5 : 6-*Di*-O-*iso*propylidene-D-*glucofuranose.*

By D. C. C. SMITH.

PHOSPHORUS PENTACHLORIDE converts 1 : 2-5 : 6-di-*O*-*iso*propylidene-D-glucofuranose into a monochloromonodeoxy-di-*O*-*iso*propylidene hexose which was assigned the structure of 3-chloro-3-deoxy-1 : 2-5 : 6-di-*O*-*iso*propylidene-D-glucofuranose.[1] However this product is dextrorotatory, whereas derivatives of 1 : 2-5 : 6-di-*O*-*iso*propylidene-D-glucofuranose are all lævorotatory,[2,3] and hydrolysis yielded a monochloromonodeoxyhexose whose constants {m. p. 135—136°, $[\alpha]_D^{20}$ +92·5° ⟶ +46·6° (equil.)}, while not agreeing with those published for 3-chloro-3-deoxy-D-glucose [3] (m. p. 155—156°, $[\alpha]_D^{20}$ +64·1°), are reasonably close to those published for 6-chloro-6-deoxy-D-glucose [4] {m. p. 135—136°, $[\alpha]_D^{19}$ +78° ⟶ +35° (equil.)}. The product of hydrolysis has now been characterised as 6-chloro-6-deoxy-D-glucose by periodate oxidation, and by conversion into 3 : 6-anhydro-β-D-glucofuranose.

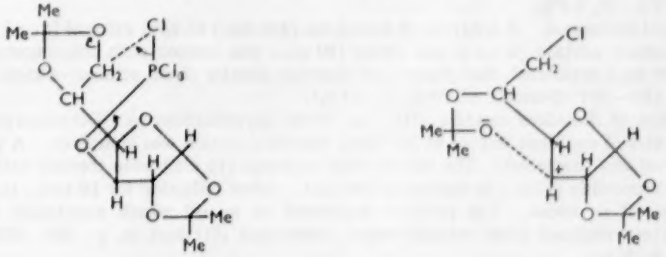
The monochloromonodeoxyhexose consumed 3·96 mols. of periodate, releasing 3·60 mols. of titratable acid. On reduction of excess of periodate with ethylene glycol, addition of standard alkali and back-titration, 5·00 mols. of alkali were found to be consumed by the oxidation products. If, instead of removing excess of periodate, the oxidation products were rendered first alkaline, then acid, 4·91 mols. of periodate were found to be consumed, 5·52 mols. of titrable acid were produced, and 0·72 mol. of formaldehyde was isolated as the the dimedone derivative. All these figures are consistent with the production of chloroacetaldehyde in the primary reaction with periodate, which was then hydrolysed by alkali to glycollaldehyde and oxidised by periodate to formaldehyde.

[1] Allison and Hixon, *J. Amer. Chem. Soc.*, 1926, **48**, 406.
[2] Vogel and Georg, "Tabellen der Zucker und ihrer Derivate," Springer, Berlin, 1931, p. 362.
[3] Newth, Overend, and Wiggins, *J.*, 1947, 10.
[4] Helferich and Bredereck, *Ber.*, 1927, **60**, 1995.

Treatment of the initial products of periodate oxidation with dimedone gave a derivative (I), $C_{18}H_{24}O_4$, m. p. 183—187°. Refluxing with hydrochloric acid in ethanol converted this into an isomeric compound (II), m. p. 231—233°. Chloroacetal and dimedone also yielded the first isomer (I). Glycollaldehyde and dimedone yielded the second isomer (II). Titration with alkali showed that isomer (I) possesses two β-keto-enol groups, whereas isomer (II) possesses one β-keto-enol group. Comparison of their ultraviolet absorption spectra in weakly acid and in alkaline solution confirmed this conclusion. Isomers (I) and (II) must therefore have the annexed structures.

This identifies chloroacetaldehyde as a product of periodate oxidation of the sugar, and so the latter must have the structure of a 6-chloro-6-deoxyhexose. Confirmation of the *gluco*-configuration was obtained by converting the 6-chloro-6-deoxyhexose into a mixture of anomeric glycosides. This was oxidised by periodate, consuming 2·0 mols. of oxidant and releasing 1·0 mol. of titratable acid, so indicating a pyranoside structure. The glycoside was hydrolysed to a crystalline but extremely hygroscopic methyl anhydrohexopyranoside. Hydrolysis of this afforded 3 : 6-anhydro-β-D-glucofuranose, m. p. 122—123°, $[\alpha]_D^{20}$ +53·8° (cf. ref. 5). Since this sequence of reactions does not involve a change of configuration at any of the asymmetric centres,[6] the 6-chloro-6-deoxyhexose must be 6-chloro-6-deoxy-D-glucose.

Migration of the *iso*propylidene group from the 5 : 6- to the 3 : 5-position can be pictured as resulting from an $S_Ni'$ mechanism of chlorination.



*Experimental.—Chlorination of* 1 : 2-5 : 6-*di*-O-*isopropylidene*-D-*glucofuranose.* Phosphorus pentachloride (50 g.), sodium carbonate (freshly ignited; 40 g.), and light petroleum (0·5 l.; b. p. 80—100°; purified over concentrated sulphuric acid, washed, distilled, and dried) were stirred for 1 hr., protected from moisture. Then 1 : 2-5 : 6-di-*O*-*iso*propylidene-D-glucofuranose (40 g.) and sodium carbonate (freshly ignited; 40 g.) were added, and the whole was stirred vigorously at room temperature for 2 hr. and at 60° for 2 hr. The light petroleum was decanted, shaken with 15% aqueous sodium hydroxide (1 l.) for 12 hr., washed, and evaporated to a syrup (9·5 g.). This was passed in benzene through alumina (Peter Spence, "H") and evaporated, giving 6-*chloro*-6-*deoxy*-1 : 2-3 : 5-*di*-O-*isopropylidene*-D-*glucofuranose* (4·0 g.), b. p. (short-path) 120°/0·05 mm., $n_D^{20}$ 1·4734, $[\alpha]_D^{20}$ +35·4° (*c* 1·95 in MeOH) (Found: C, 52·0; H, 6·7; Cl, 13·0. $C_{12}H_{19}O_5Cl$ requires C, 51·8; H, 6·9; Cl, 12·7%).

6-*Chloro*-6-*deoxy*-D-*glucose.* 6-Chloro-6-deoxy-1 : 2-3 : 5-di-*O*-*iso*propylidene-D-glucofuranose (4·58 g.) in methanol (100 c.c.) and N-sulphuric acid (30 c.c.) was refluxed for 9 hr. The solution was freed from acid with Amberlite resin IR-4B, and evaporated to a glass (3·19 g.). When moistened with acetone and kept, this eventually crystallised. Recrystallised from ethanol–ether at 5°, it gave 6-chloro-6-deoxyglucose as needles, m. p. 135—136°, $[\alpha]_D^{20}$ +92·5° ⟶ +46·6°

[5] Fischer and Zach, *Ber.*, 1912, **45**, 456.
[6] Peat, *Ann. Reports*, 1939, **36**, 261.

(equil.) (*c* 7·6 in $H_2O$) (Found : C, 36·5; H, 5·8. Calc. for $C_6H_{11}O_5Cl$ : C, 36·3; H, 5·6%), whose *phenylosazone* had m. p. 167—171° (Found : C, 57·4; H, 5·6; N, 14·4; Cl, 9·9. $C_{18}H_{21}O_3N_4Cl$ requires C, 57·4; H, 5·6; N, 14·9; Cl, 9·4%).

*Periodate oxidation of 6-chloro-6-deoxy-D-glucose.* A solution of 6-chloro-6-deoxy-D-glucose (136 mg.) in 0·090M-sodium metaperiodate (50 c.c.) was kept at room temperature. Excess of periodate was determined by Barneby's method.[7] Free acid was determined after reducing excess of periodate with ethylene glycol, either by titrating aliquots with 0·01N-sodium hydroxide to the end-point of screened methyl-red (forward titration), or by treating aliquot parts (1 c.c.) with 0·01N-sodium hydroxide (10 c.c.) and then titrating them with 0·01N-sulphuric acid to the end-point of screened methyl-red (backward titration).

| | | | | | |
|---|---|---|---|---|---|
| Time (min.) | 5 | 25 | 65 | 115 | 330 |
| Periodate consumed (mols.) | 2·66 | 3·35 | 3·77 | 3·94 | 3·96 |
| Time (min.) | 15 | 37 | 65 | 115 | 480 |
| Acid released (forward titrn.) (equiv.) | 2·04 | 2·65 | 3·01 | 3·30 | 3·60 |
| Time (min.) | 10 | 40 | 65 | 115 | 330 |
| Acid released (backward titrn.) (equiv.) | 3·48 | 4·31 | 4·70 | 4·94 | 5·00 |

Aliquot parts (1 c.c.) of the solution were treated with 0·1N-sodium hydroxide (1 c.c.), kept 1 hr., then treated with 0·1N-sulphuric acid (1 c.c.); periodate consumed (estimated as above) was 4·91 mols.; acid released (forward titration) was 5·52 mols.; formaldehyde recovery (method of Reeves [8]) was 0·72 mol.

*Preparation of keten–dimedone complex* (I). (*a*) From 6-chloro-6-deoxy-D-glucose. A solution of 6-chloro-6-deoxy-D-glucose (104 mg.) in water (5 c.c.) and 0·30M-sodium metaperiodate (10 c.c.) was kept for 4 hr. at room temperature, then treated with N-hydrochloric acid (10 c.c.), then 1·2N-disodium hydrogen arsenite (10 c.c.), followed after disappearance of the iodine, by M-sodium acetate (20 c.c.) and dimedone (400 mg.) in 95% ethanol (5 c.c.). After 12 hr. the precipitate was filtered off, washed with water, and dried (52 mg.). The *compound* (I) formed needles (from ethanol–water), m. p. 183—187° (Found : C, 71·1; H, 8·3. $C_{18}H_{24}O_4$ requires C, 71·0; H, 7·9%).

(*b*) From chloroacetal. A solution of dimedone (300 mg.) in 95% ethanol (4 c.c.), acetic acid (1 c.c.), M-sodium acetate (5 c.c.), and water (20 c.c.) was heated with chloroacetal (0·15 g.). Crystals (200 mg.) separated, this compound forming needles (from ethanol–water), m. p. and mixed m. p. 183—187° (Found : C, 70·5; H, 8·1%).

*Preparation of dimedone complex* (II). (*a*) From glycollaldehyde. Dihydroxymaleic acid (1·5 g.) in water (5 c.c.) was heated at 80° until decarboxylation was complete. A yellow solid was filtered off and discarded. The filtrate and washings (10 c.c.) were treated with dimedone (2·5 g.) and piperidine (1 c.c.) in methanol (40 c.c.). After refluxing for 10 min., the methanol was distilled off *in vacuo*. The product separated as an oil which eventually crystallised (1·35 g.). Recrystallised from ethanol–water, *compound* (II) had m. p. 231—233° (Found : C, 70·9; H, 8·1%).

(*b*) From keten–dimedone complex (I). The compound (I) (100 mg.), 95% ethanol (3 c.c.), and concentrated hydrochloric acid (3 drops) were refluxed for 10 min., diluted with water, and extracted with ether. The extract was washed and evaporated to a glass (100 mg.). Recrystallised from ethanol–water, compound (II) had m. p. and mixed m. p. 231—233°.

*Estimation of β-keto-enol groups.* (*a*) By potentiometric titration. The amount of alkali neutralised by each dimedone derivative at pH 10 was : compound (I) 2·00 equivs., (II) 1·01 equivs.

(*b*) By ultraviolet light absorption. See Table.

| | Acidified EtOH | | 0·2N-Sodium hydroxide | |
|---|---|---|---|---|
| Solvent : | $\lambda_{max.}$ | log ε | $\lambda_{max.}$ | log ε |
| Acetaldehyde–dimedone compound | 259 | 4·36 | 285 | 4·69 |
| Compound (I) | 261 | 4·37 | 285 | 4·63 |
| Compound (II) | 267 | 4·42 | 287 | 4·52 |

*Methyl 6-chloro-6-deoxy-αβ-D-glucopyranoside.* 6-Chloro-6-deoxy-D-glucose (1·36 g.) and 2% hydrogen chloride in methanol were refluxed for 8 hr., neutralised with silver carbonate,

[7] Barneby, *J. Amer. Chem. Soc.*, 1916, **38**, 330.
[8] Reeves, *ibid.*, 1941, **63**, 1476.

filtered, and evaporated to a syrup (1·19 g.) (Found : C, 39·7; H, 6·1. Calc. for $C_7H_{13}O_6Cl$ : C, 39·6; H, 6·2%). A solution of this glycoside (97·9 mg.) in 0·0636M-sodium metaperiodate (25 c.c.) was kept at room temperature.

| | | | | | | |
|---|---|---|---|---|---|---|
| Time (min.) ..... | 4 | 20 | 55 | 137 | 270 | 1202 |
| Periodate consumed (mols.) ..... | 0·12 | 0·61 | 1·23 | 1·61 | 1·84 | 2·05 |
| Time (min.) ..... | 4 | 21 | 58 | 139 | 277 | 1202 |
| Acid released (equiv.) ..... | 0·08 | 0·25 | 0·50 | 0·72 | 0·84 | 1·00 |

*Methyl* 3 : 6-*anhydro*-αβ-D-*glucopyranoside*. Methyl 6-chloro-6-deoxy-αβ-D-glucopyranoside (0·97 g.), ethanol (20 c.c.), and N-sodium hydroxide (10 c.c.) were refluxed for 3 hr., neutralised with carbon dioxide, evaporated, and extracted with boiling acetone. The extract, on evaporation, gave a syrup (0·46 g.), b. p. (short-path) 90°/0·05 mm. The distillate crystallised but was very deliquescent (Found : C, 47·7; H, 6·9. Calc. for $C_7H_{12}O_5$ : C, 47·7; H, 6·9%).

3 : 6-*Anhydro*-β-D-*glucofuranose*. Methyl 3 : 6-anhydro-αβ-D-glucopyranoside (0·36 g.) and 0·1N-sulphuric acid (5 c.c.) were heated at 100° for 3 hr. The solution was freed from acid with Amberlite resin IR-4B, and evaporated, leaving a crystalline residue (0·27 g.) which formed needles, m. p. 122—123°, $[\alpha]_D^{20}$ +53·8° (*c* 1·9 in $H_2O$), from ethanol–ethyl acetate–light petroleum (Found : C, 44·5; H, 6·2. Calc. for $C_6H_{10}O_5$ : C, 44·4; H, 6·2%).

THE UNIVERSITY, MANCHESTER, 13. [*Received, October 11th*, 1955.]

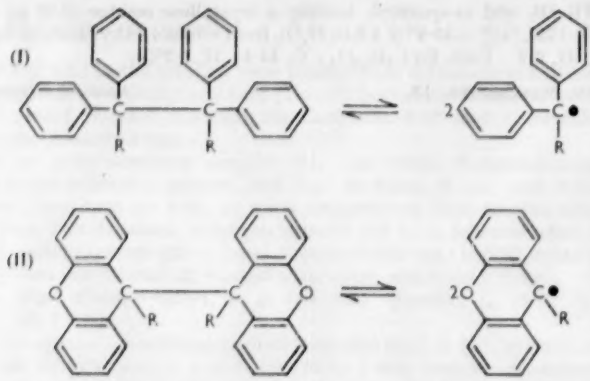
# *Chemical Effects of Steric Strains.*

A Centenary Lecture delivered at Burlington House on June 2nd, 1955.

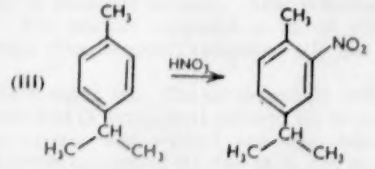
By Herbert C. Brown.

Steric effects as a factor in chemical behaviour appear to have originated with Kehrmann's observations on the chemical inertness of quinones containing *ortho*-substituents.[1a] Investigation of similar phenomena in the esterification of hindered aromatic acids was later pursued with considerable success by Victor Meyer and numerous contemporary workers.[1b, c]

With the years, evidence accumulated that steric effects were of widespread importance in chemical behaviour. Thus Conant and his co-workers noted that the tendency of the dialkyltetraphenylethanes (I) and the dialkyldixanthyls (II) to dissociate into free radicals increased with the increasing bulk of the alkyl groups (Me < Et < Pr$^i$ < Bu$^t$).[2a, b] Likewise the discovery




of diphenyl isomerism opened up an entire area of chemistry resulting from steric forces.[2c, d] Certain peculiarities in *ortho*-substitution led Holleman to suggest that steric effects were an important factor in aromatic substitution,[2e] and Le Fèvre later utilized this explanation to account for the observed directive effects in the nitration and sulphonation of *p*-cymene (III) :[2f]




Moreover, Polanyi utilized the concept to account for the decreasing rates of the reactions of iodide ion with methyl, ethyl, and *iso*propyl chloride.[2g, h]

The immense success achieved by the electronic theory in the 1930's led to the attempt to account for all chemical behaviour in terms of electronic effects. For example, Wheland suggested that the increased dissociation of the dialkyltetraphenylethanes (I) and the dialkyldixanthyls (II) with increasing bulk of the alkyl groups might be due to the increased possibility for resonance in which the alkyl groups participate.[3a] Likewise it was suggested that the directive effects in *p*-cymene were due, not to the relative steric requirements of the methyl and

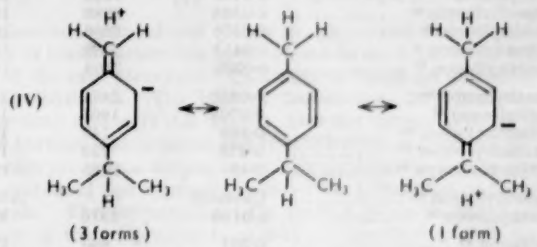
[1] (*a*) Kehrmann, *J. prakt. Chem.*, 1889, **40**, 257; (*b*) Meyer, *Ber.*, 1894, **27**, 510; (*c*) Cohen, "Organic Chemistry," Edward Arnold and Co., London, 1928, 5th edn., Vol. I, Chapt. 5.

[2] (*a*) Conant and Bigelow, *J. Amer. Chem. Soc.*, 1928, **50**, 2041; (*b*) Conant, Small, and Sloan, *ibid.*, 1926, **48**, 1743; (*c*) Turner and Le Fèvre, *Chem. and Ind.*, 1926, **45**, 831; (*d*) Adams and Yuan, *Chem. Rev.*, 1933, **12**, 261; (*e*) Holleman, *ibid.*, 1924, **1**, 187; (*f*) Le Fèvre, *J.*, 1933, 977, 980; 1934, 1501; (*g*) Meer and Polanyi, *Z. phys. Chem.*, 1932, **19**, *B*, 164; (*h*) Evans, *Trans. Faraday Soc.*, 1946, **42**, 719.

[3] (*a*) Wheland, *J. Chem. Phys.*, 1934, **2**, 474; (*b*) Baker and Nathan, *J.*, 1935, 1844; (*c*) Ingold, *Chem. Rev.*, 1934, **15**, 225; (*d*) Hinshelwood, Laidler, and Timm, *J.*, 1938, 848.

*iso*propyl groups, but instead to their relative ability to participate in hyperconjugation (IV).[3b] Similarly, the decreased reactivity toward bimolecular displacement reactions in the series, MeX, EtX, $Pr^{i}X$, and $Bu^{t}X$, was attributed to the polar effect of the alkyl substituents.[3c, d]

As a result of these developments there had arisen by 1940 a widespread scepticism as to the importance of steric effects in chemical behaviour. The excellent book published in 1940 by Professor Hammett played a major rôle in the development of physical organic chemistry in the United States. Yet steric effects are conspicuously absent from its discussions.[4a] Similarly, in the book by Professor Remick, published in 1944, there appears no discussion of the rôle of steric effects in organic theory.[4b] Perhaps the prevalent view is best expressed in the words of a textbook published at this time: "Steric hindrance . . . has become the last refuge of the puzzled organic chemist."[4c] It was in this atmosphere that our programme for investigating the rôle of steric effects in chemical behaviour was undertaken.




It was apparent that if steric effects were again to receive serious consideration as a major factor in chemical behaviour there would be required a technique of study which could provide quantitative data on the magnitudes of the steric effects in the systems under investigation. The dissociation of molecular addition compounds offered promise of providing such a tool and their study was therefore undertaken.

The original studies utilized essentially qualitative competition experiments.[5] Their success encouraged the development of quantitative methods of determining thermodynamic data for the dissociation of addition compounds. The main reliance has been on the measurement of the dissociation constants for the gaseous systems over a range of temperatures.[6] More recently calorimetric procedures have been utilized for addition compounds which cannot be handled as gases.[7] The results of these studies are summarized in Tables 1 and 2.

Space will not permit a review of the various studies which were made in an effort to test the postulate that steric effects were a major factor in chemical behaviour. We shall be content therefore to outline two studies which will serve to illustrate the utility of the approach based on molecular addition compounds.

The heat of dissociation of ammonia–trimethylboron is 13·75 kcal./mole. This rises to 17·64 for methylamine– and to 18·0 for ethylamine–trimethylboron. These increases are readily attributed to the inductive effects of the methyl and the ethyl groups. The heat of dissociation of trimethylamine–trimethylboron is 17·62, not significantly different from that of the methylamine derivative. However, triethylamine–trimethylboron is a highly unstable substance with a heat of dissociation in the neighbourhood of 10 kcal./mole. How can we account for the observation that one ethyl group has about the same effect as one methyl group, that three methyl groups are not significantly different than one methyl group, but that three ethyl groups bring about an enormous decrease in the stability of the addition compound?

A possible explanation is that the steric requirements of three ethyl groups are far larger than those of three methyl groups and therefore result in much larger steric strains in the addition compound. Indeed, an examination of the molecular models suggests that it is possible

[4] (*a*) Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., New York, N.Y., 1940; (*b*) Remick, "Electronic Interpretation of Organic Chemistry," John Wiley and Sons, Inc., New York, N.Y., 1944; (*c*) Ray, "Organic Chemistry," J. B. Lippincott Co., New York, N.Y., 1941, p. 522.

[5] Qualitative studies of steric strains: Brown, Schlesinger, and Cardon, *J. Amer. Chem. Soc.*, 1942, **64**, 325; Brown, *ibid.*, 1945, **67**, 374, 378, 503, 1452; Brown and Pearsall, *ibid.*, p. 1765; Brown and Johannesen, *ibid.*, 1950, **72**, 2934; Brown and Sujishi, *ibid.*, 1948, **70**, 2793.

[6] Gaseous dissociation techniques: (*a*) Brown, Taylor, and Gerstein, *ibid.*, 1944, **66**, 431; (*b*) Brown and Gerstein, *ibid.*, 1950, **72**, 2923.

[7] Calorimetric techniques: (*a*) Brown and Horowitz, *ibid.*, 1955, **77**, 1730; (*b*) Brown and Gintis, *ibid.*, in the press.

TABLE 1. *Thermodynamic data for the dissociation of molecular addition compounds in the gaseous phase.*

| Compound | $K_{100}$ | $\Delta F°_{100}$ | $\Delta H$ | $\Delta S$ |
|---|---|---|---|---|
| Ammonia–trimethylboron [5a] | 4·6 | −1134 | 13·75 | 39·9 |
| Methylamine–trimethylboron [5a] | 0·0350 | 2472 | 17·64 | 40·6 |
| Dimethylamine–trimethylboron [5a] | 0·0214 | 2885 | 19·26 | 43·6 |
| Trimethylamine–trimethylboron [5a] | 0·472 | 557 | 17·62 | 45·7 |
| Ethylamine–trimethylboron [5b] | 0·705 | 1965 | 18·00 | 43·0 |
| Diethylamine–trimethylboron [5b] | 1·22 | − 147 | 16·31 | 44·1 |
| Triethylamine–trimethylboron [5b] | Unstable | — | ~10 | — |
| Methylamine–trimethylboron [5a] | 0·0350 | 2472 | 17·64 | 40·6 |
| Ethylamine–trimethylboron [5b] | 0·0705 | 1965 | 18·00 | 43·0 |
| *n*-Propylamine–trimethylboron [5c] | 0·0598 | 2088 | 18·14 | 43·0 |
| *n*-Butylamine–trimethylboron [5c] | 0·0470 | 2266 | 18·41 | 43·2 |
| *n*-Pentylamine–trimethylboron [5c] | 0·0415 | 2359 | 18·71 | 43·9 |
| *n*-Hexylamine–trimethylboron [5c] | 0·0390 | 2404 | 18·53 | 43·2 |
| Methylamine–trimethylboron [5a] | 0·0350 | 2472 | 17·64 | 40·6 |
| Ethylamine–trimethylboron [5b] | 0·0705 | 1965 | 18·00 | 43·0 |
| *iso*Propylamine–trimethylboron [5d] | 0·368 | 740 | 17·42 | 44·7 |
| *sec.*-Butylamine–trimethylboron [5d] | 0·873 | 732 | 17·26 | 44·3 |
| *tert.*-Butylamine–trimethylboron [5d] | 9·46 | −1665 | 12·99 | 39·3 |
| Triethylamine–trimethylboron [5b] | Unstable | — | ~10 | — |
| Quinuclidine–trimethylboron [5e] | 0·0196 | 2916 | 19·94 | 45·6 |
| Pyridine–trimethylboron [5f] | 0·301 | 890 | 17·00 | 43·2 |
| 2-Picoline–trimethylboron [5f] | Unstable | — | ~10 | — |
| 3-Picoline–trimethylboron [5f] | 0·138 | 1468 | 17·81 | 43·9 |
| 4-Picoline–trimethylboron [5f] | 0·105 | 1671 | (19·40) | (47·5) |
| Dimethylamine–trimethylboron [5a] | 0·0214 | 2885 | 19·26 | 43·6 |
| Ethyleneimine–trimethylboron [5g] | 0·0284 | 2640 | 17·59 | 40·1 |
| Trimethyleneimine–trimethylboron [5g] | 0·000332 | 5960 | 22·48 | 44·3 |
| Pyrrolidine–trimethylboron [5g] | 0·00350 | 4190 | 20·43 | 43·5 |
| Piperidine–trimethylboron [5g] | 0·0210 | 2864 | 19·65 | 45·0 |
| Methylphosphine–trimethylboron [5h] | Too highly dissociated to be measured | | | |
| Dimethylphosphine–trimethylboron [5h] | 9·8 | −1690 | 11·41 | 35·1 |
| Trimethylphosphine–trimethylboron [5h] | 0·128 | 1525 | 16·47 | 40·0 |
| Methylphosphine–boron trifluoride [5i] | Too highly dissociated to be measured | | | |
| Dimethylphosphine–boron trifluoride [5i] | 10·5 | −1740 | 14·7 | 44·1 |
| Trimethylphosphine–boron trifluoride [5i] | 0·0669 | 1986 | 18·9 | 45·3 |

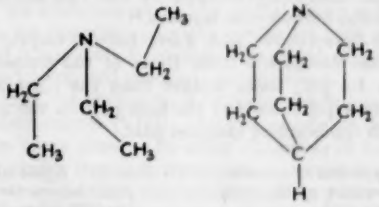
TABLE 2. *Calorimetric heats of reaction of pyridine bases with reference acids.*

| Compound | $pK_a$ [12d] | Heats of reaction, $-\Delta H$ (kcal./mole) [a] $CH_3{\cdot}SO_3H$ [12a] | $\frac{1}{2}(BH_3)_2$ [12d] | $BF_3$ [12f] | $BMe_3$ [12h] |
|---|---|---|---|---|---|
| Pyridine | 5·17 | 17·1 | 17·9 | 25·0 | 15·3 |
| 2-Picoline | 5·97 | 18·3 | 17·2 | 23·3 | 9·95 |
| 2-Ethylpyridine | 5·97 | 18·2 | 16·9 | 22·7 | 9·0 |
| 2-*iso*Propylpyridine | 5·83 | 18·1 | 16·7 | 21·7 | 7·7 |
| 2-*tert.*-Butylpyridine | 5·76 | 18·0 | 12·1 | 14·8 | <0 |
| 3-Picoline | 5·68 | 17·8 | 18·2 | 25·3 | 15·6 |
| 3-Ethylpyridine | 5·70 | 18·1 | — | 25·2 | — |
| 3-*iso*Propylpyridine | 5·72 | 18·0 | — | 25·3 | — |
| 3-*tert.*-Butylpyridine | 5·82 | 18·2 | — | 25·6 | — |
| 4-Picoline | 6·02 | 18·4 | 18·5 | 25·5 | 15·9 |
| 4-Ethylpyridine | 6·02 | 18·3 | — | 25·3 | — |
| 4-*iso*Propylpyridine | 6·02 | 18·4 | — | 25·4 | — |
| 4-*tert.*-Butylpyridine | 5·99 | 18·3 | — | 25·3 | — |
| 2 : 6-Lutidine | 6·75 | 19·5 | 16·3 | 17·5 | <0 |

[a] At 25° in nitrobenzene solution : Base (soln.) + Acid (soln.) = Product (soln.).

[5] Dissociation data for gaseous addition compounds : (*a*) Brown, Bartholomay, jun., and Taylor, *J. Amer. Chem. Soc.*, 1944, **66**, 435; (*b*) Brown and Taylor, *ibid.*, 1947, **69**, 1332; (*c*) Brown, Taylor, and Sujishi, *ibid.*, 1951, **73**, 2464; (*d*) Brown and Barbaras, *ibid.*, 1953, **75**, 6; (*e*) Brown and Sujishi, *ibid.*, 1948, **70**, 2878; (*f*) Brown and Barbaras, *ibid.*, 1947, **69**, 1137; (*g*) Brown and Gerstein, *ibid.*, 1950, **72**, 2926; (*h*) Sujishi, Thesis, Purdue, 1949; (*i*) Fletcher, Thesis, Purdue, 1952.

to rotate only two of the three ethyl groups out of the path of the trimethylboron molecule—the third must project in such a way as to interfere with the adding molecule (V).

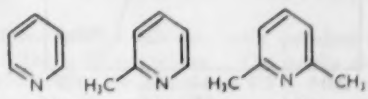


(V) (VI)

To test this explanation quinuclidine (VI) was synthesised and tested. In this molecule the third ethyl group of triethylamine has been rotated to the back of the amine and effectively held there as part of the cage structure. Quinuclidine forms an exceedingly stable addition compound with trimethylboron. The heat of dissociation, 20·0 kcal./mole, is the highest observed for any tertiary amine (Table 1). It does not appear possible to account for the enormous difference between quinuclidine and triethylamine in terms of polar effects, whereas the steric interpretation provides a simple, reasonable explanation for the observed phenomena.

As a second illustration of this approach, consider the interaction of the pyridine bases with various reference acids. The introduction of a methyl group in the 3-position of pyridine results in an increase of the $pK_a$ value from 5·17 for pyridine to 5·68 for 3-picoline. This increase is attributed to the inductive effect of the methyl group. 3-Picoline likewise exhibits a consistent increase over pyridine in its heat of reaction with methanesuphonic acid, diborane, boron trifluoride, and trimethylboron, all in nitrobenzene solution (Table 2).

The effects of the methyl group in the 2-position do not exhibit the same consistency. The $pK_a$ value and the heats of reaction with methanesulphonic acid show a regular increment with one and then two methyl groups in the series, pyridine, 2-picoline, 2 : 6-lutidine, suggesting that we are observing the polar effects of the methyl groups uncomplicated by their steric requirements. However, with borine, boron trifluoride, and trimethylboron, the heats of reaction exhibit a decrease rather than the expected increase. Moreover, the discrepancy increases quite sharply with the increasing steric requirements of the reference acid.



| | | | |
|---|---|---|---|
| $pK_a$ | 5·17 | 5·97 | 6·75 |
| $-\Delta H$, $Me{\cdot}SO_3H$ | 17·1 | 18·3 | 19·5 |
| $-\Delta H$, $\frac{1}{2}(BH_3)_2$ | 17·9 | 17·2 | 16·3 |
| $-\Delta H$, $BF_3$ | 25·0 | 23·3 | 17·5 |
| $-\Delta H$, $BMe_3$ | 15·3 | 9·95 | <0 |

The phenomenon is represented graphically in Fig. 1. It does not appear possible to account for these results in terms of any polar effects of the different reference acids. However, a simple explanation is afforded in terms of the steric requirements of the acids. In the case of the proton in water ($pK_a$) and methanesulphonic acid, the steric requirements are so small that a regular increase in base strength results from the presence of one and two methyl groups in the pyridine base. Borine is sufficiently large to cause steric interaction with the methyl groups in the 2-position. The resulting strains cause a small decrease in the heat of reaction of diborane with 2-picoline and a larger decrease in the case of 2 : 6-lutidine. With the increasing steric requirements of boron trifluoride and trimethylboron, the strains become much larger, and the observed heats of reaction drop sharply below those to be expected. Finally, trimethylboron fails to add to 2 : 6-lutidine in spite of the fact that it is a stronger base than pyridine itself.

It was of interest to synthesize a number of pyridine bases with bulky alkyl substituents in the 2- and the 6-position in order to ascertain whether steric strain involving addition of a proton could be realised. The final member of this group, 2 : 6-di-*tert.*-butylpyridine, possesses highly interesting and unusual properties. It reacts with hydrogen chloride, but not with boron trifluoride, thus providing a simple method of distinguishing between protonic and Lewis acids. The base fails to react with methyl iodide. On the other hand, in contrast to less sterically

hindered pyridine bases, it reacts readily with sulphur trioxide (at $-10°$ in liquid sulphur dioxide) to form a nuclear-substituted sulphonic acid, presumably the 4-derivative. Apparently, with addition to the nitrogen atom effectively blocked by the two *tert.*-butyl groups, the electrophilic reagent readily attacks the heterocyclic nucleus.[8c]

The $pK_a$ values of 2 : 6-di*iso*propyl- and 2-*tert.*-butyl-6-*iso*propyl-pyridine are perfectly regular and agree with values calculated from those of the simpler derivatives. However, 2 : 6-di-*tert.*-butylpyridine is 1·4 $pK_a$ units weaker than the calculated value (Fig. 2). This result suggests that the steric requirements of the lone pair on the nitrogen atom must be less than those of the proton with the bonding electron pair.

FIG. 1. *Relative strengths of pyridine, 2-picoline, and 2 : 6-lutidine as a function of the steric requirements of the reference acid.*

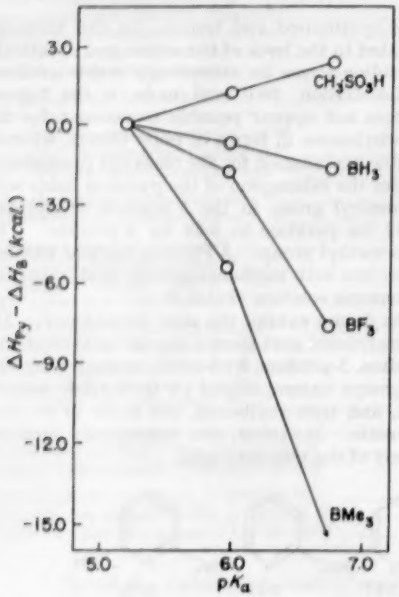
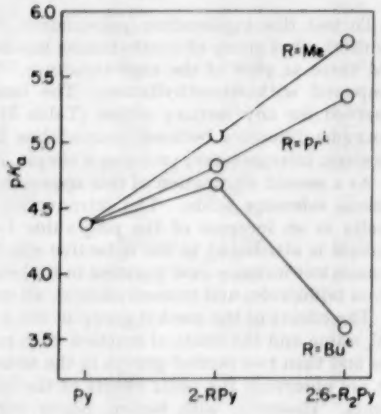
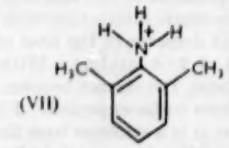



FIG. 2. *Effect of alkyl groups in the 2 : 6-positions on the strength of pyridine bases (in 50% ethanol).*

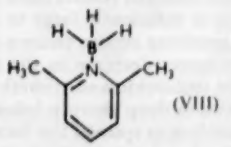



Methyl groups in the *meta-* or *para*-position of aniline bases increase the base strength. This change is, of course, consistent with the postulated inductive effect of alkyl groups. In the *ortho*-position, however, such groups considerably decrease the base strength.[8c] Moreover, *tert.*-butyl groups, as in *o-tert.*-butyl- and 2 : 6-di-*tert.*-butyl-aniline, bring about a much sharper decrease in the strength of the substituted bases.[8d]

The geometrical requirements of 2 : 6-dimethylanilinium ion (VII) must be very similar to those of 2 : 6-lutidine–borine (VIII), a molecule in which strains of 2·8 kcal./mole are indicated




(VII)




(VIII)

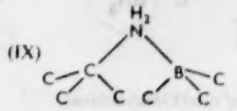
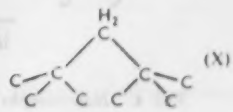
(Table 2). Consequently, it appears reasonable to attribute the unexpected weakness of *o*-toluidine and 2 : 6-xylidine to the increase in the steric requirements of the $-NH_2$ group in its

[8] Steric requirements of the proton: (*a*) Brown and Kanner, *J. Amer. Chem. Soc.*, 1953, **75**, 3865; (*b*) Brown and Cahn, *ibid.*, 1950, **72**, 2939; (*c*) Thomson, *J.*, 1946, 1113; (*d*) L. P. Wepster (Delft Univ.) unpublished data.

conversion into the $-NH_3^+$ group. Strains resulting from the conflicting steric requirements of the $-NH_3^+$ group and the methyl groups in the *ortho*-positions would enhance the tendency for the loss of a proton from the anilinium ion. The $\geq\overset{+}{N}-H$ group in the pyridinium ion and the $-NH_3^+$ in the anilinium ion are doubtless strongly associated with a sheath of solvating water molecules. To what extent it is necessary to consider this sheath of water molecules in estimating the steric requirements of the protonated groups is not yet clear.

It was suggested above that the strains in 2 : 6-dimethylanilinium ion (VII) are probably similar to those in 2 : 6-lutidine–borine (VIII). Unfortunately, it does not appear possible at present to test this point in these cases. In other examples it has been found possible to demonstrate the existence of similar strains in molecules of related structures.[10]

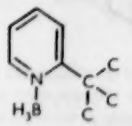
For example, *n*-butylamine and *tert.*-butylamine are primary amines of quite similar strengths. Consequently, it would be predicted that their addition compounds with trimethylboron should exhibit similar stabilities. In actual fact *tert.*-butylamine–trimethylboron, with a heat of dissociation of 13·0, is far less stable than *n*-butylamine–trimethylboron, with a heat of dissociation of 18·4 kcal./mole.[10b] The discrepancy of 5·4 kcal. is attributed to steric strain arising from the crowding of the two bulky groups attached to the common nitrogen atom (IX).

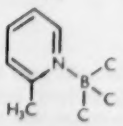
(IX)   (X)

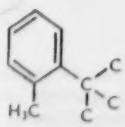
The structure of di-*tert.*-butylmethane (X) is quite similar to that of the addition compound. The covalent radius of nitrogen is slightly smaller than that of carbon, while that of boron is slightly larger. With the boron-to-nitrogen distance almost identical with the carbon-to-carbon distance, it is of interest to compare the strain in the hydrocarbon with that in the addition compound.

As a result of the very precise work carried out at the National Bureau of Standards in Washington on the combustion of hydrocarbons it is now possible to calculate the heat of combustion of any simple hydrocarbon with considerable precision. The heat of combustion thus calculated for di-*tert.*-butylmethane turns out to be 5·2 kcal./mole smaller than the experimental value.[10a] It appears, therefore, that the strain present in the hydrocarbon is essentially identical with that present in the addition compound.

Recently we have determined the heat of reaction of diborane with pyridine and the *tert.*-butylpyridines, and of trimethylboron and the methylpyridines. The results show that 2-*tert.*-butylpyridine–borine (XI) is less stable than the other isomers by 6·3 ± 0·2 kcal./mole, while 2-picoline–trimethylboron (XII) is less stable than the other isomers by 5·9 ± 0·2


(XI)


(XII)


(XIII)

kcal./mole. The heats of combustion of the isomeric *tert.*-butyltoluenes have recently been measured by Mr. Prosen and his associates at the Bureau of Standards. They find the *ortho*-isomer (XIII) to be less stable than the *meta*- and the *para*-derivative by 5·6 ± 0·8 kcal./mole. Thus, within the experimental error of the measurements, these three related systems possess identical strains of approximately 5·9 ± 0·2 kcal./mole.

It is therefore reasonable to assume that molecules which are closely related structurally in this way will possess very similar steric strains. The term " homomorph " has been proposed for molecules which have similar molecular dimensions.

[10] Strained homomorphs: (*a*) Brown, Barbaras, Berneis, Bonner, Johannesen, Grayson, and Nelson, *J. Amer. Chem. Soc.*, 1953, **75**, 1; (*b*) Brown and Barbaras, *ibid.*, p. 6; (*c*) Brown and Berneis, *ibid.*, p. 10; (*d*) Brown and Bonner, *ibid.*, p. 14; (*e*) Brown and Johannesen, *ibid.*, p. 16; (*f*) Brown and Grayson, *ibid.*, p. 20; (*g*) Brown and Nelson, *ibid.*, p. 24.

Typical homomorphic systems are shown in Figs. 3, 4, 5. It has proved possible to examine typical structures in each system and to follow the effects of the strain upon the chemical behaviour of the molecule. Unfortunately, space will not permit a review of these results.[10]

It was previously pointed out that the preferred substitution in the position *ortho* to methyl in *p*-cymene was attributed by Le Fèvre to the operation of steric effects.[21] However, this

FIG. 3. *Homomorphs of di-*tert.*-butylmethane.*

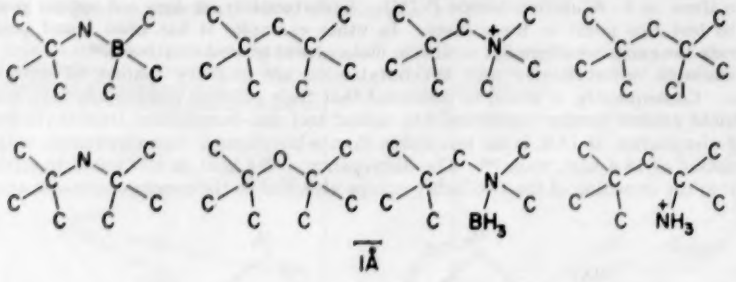
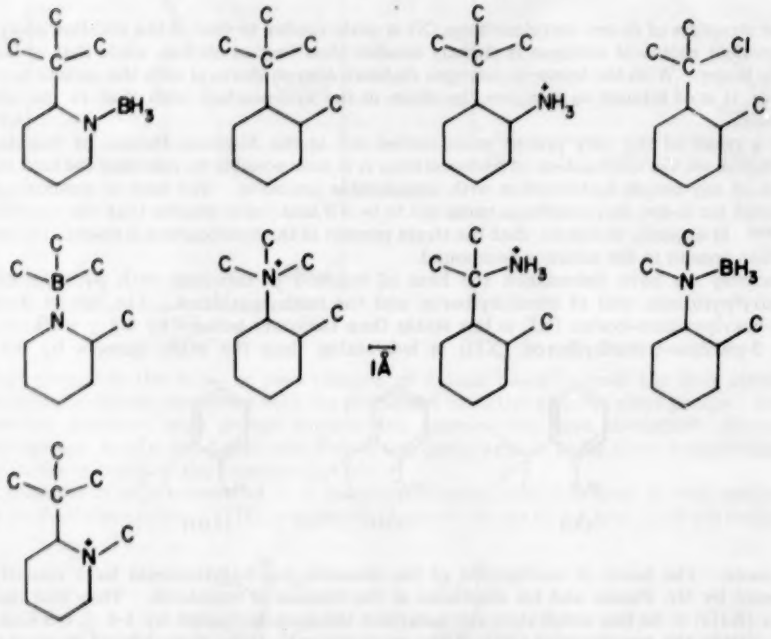


FIG. 4. *Homomorphs of* o*-*tert.*-butyltoluene.*



phenomenon has been attributed alternatively to electronic factors (IV).[35] In an attempt to resolve this difference in interpretation we examined the isomer distribution in the nitration of toluene, and ethyl-, *iso*propyl-, and *tert.*-butyl-benzene. The experimental results are summarized in Table 3.

The *meta*-position is relatively insensitive to both resonance interactions and steric factors. Consequently, it offers a satisfactory position of reference. From the observation that the *para*/*meta* ratio is sensibly constant in the series of four compounds, we may conclude that there are no *major* differences in the hyperconjugative contributions of the four alkyl groups.

FIG. 5. *Homomorphs of* 2-tert.-*butyl*-1 : 3-*dimethylbenzene.*

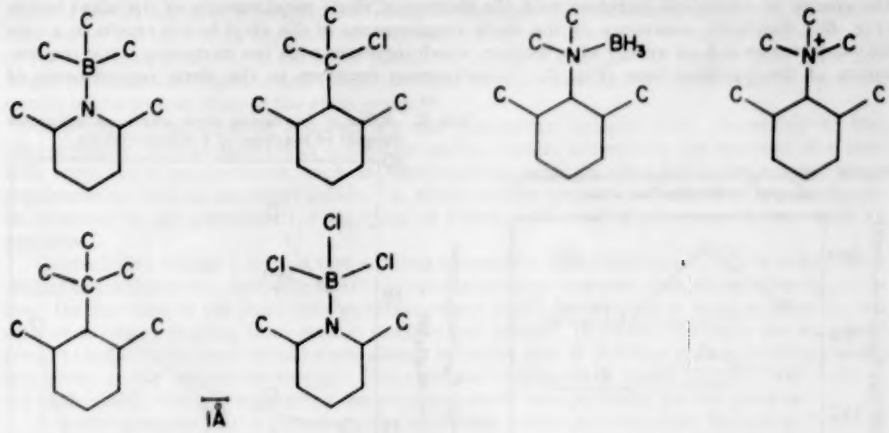
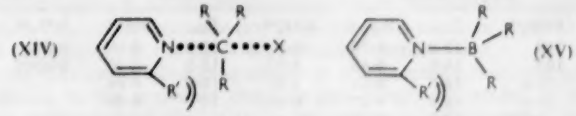


TABLE 3. *Isomer distribution in the nitration of the monoalkylbenzenes.*

| Monoalkylbenzene | Isomer distribution | | | Isomer ratio | |
|---|---|---|---|---|---|
| | *o*- | *m*- | *p*- | *o*-/*m*- | *p*-/*m*- |
| Toluene [11e] | 58·45 | 4·4 | 37·15 | 13·3 | 8·5 |
| Ethylbenzene [11b] | 45·0 | 6·5 | 48·5 | 6·9 | 7·5 |
| *iso*Propylbenzene [11b] | 30·0 | 7·7 | 62·3 | 3·9 | 8·1 |
| *tert.*-Butylbenzene [11a, d] | 15·8 | 11·5 | 72·7 | 1·37 | 6·3 |

The sharp decrease in the *ortho*/*meta* ratios cannot then be the result of differences in the hyperconjugative contributions of the alkyl groups. The decrease is therefore more plausibly ascribed to the increasing steric requirements of these groups. The results therefore provide clear evidence of the importance of the steric factor in influencing substitution in the *ortho*-position.

The transition state for a typical displacement reaction (XIV) resembles rather closely molecular addition compounds of related structures (XV) :



On this basis it appears resonable to expect that structural changes which result in an increase of strain in the addition compounds will also result in increased strain in the transition state. To test this idea we undertook a study of the reaction of a series of pyridine bases [12b] with methyl, ethyl, and *iso*propyl iodide.[12c]

As expected, the reaction rate decreases sharply from methyl to ethyl to *iso*propyl iodide with the activation energies showing corresponding increases. Introduction of an alkyl group into the 3- or 4-position results in a small increase in rate, with no significant difference for a methyl, ethyl, *iso*propyl, or *tert.*-butyl substituent. In the 2-position these substituents bring about decreases in rate and increases in activation energy which become more and more pronounced with the increasing steric requirements of the alkyl group.

[11] Steric effects in aromatic substitution : (*a*) Nelson and Brown, *J. Amer. Chem. Soc.*, 1951, **73**, 5605; (*b*) Brown and Bonner, *ibid.*, 1954, **76**, 605; (*c*) Jones and Russell, *J.*, 1947, 921; (*d*) Cohn, Hughes, Jones, and Peeling, *Nature*, 1952, **169**, 291.

[12] Steric effects in displacement reactions : (*a*) Brown and Eldred, *J. Amer. Chem. Soc.*, 1949, **71**, 445; (*b*) Brown and Murphey, *ibid.*, 1951, **73**, 3308; (*c*) Brown and Cahn, *ibid.*, 1955, **77**, 1715; (*d*) Brown and Mihm, *ibid.*, p. 1723; (*e*) Brown and Holmes, *ibid.*, p. 1727; (*f*) Brown and Horowitz, *ibid.*, pp. 1730, 1733; (*g*) Brown, Gintis, and Podall, *ibid.*, in the press; (*h*) Brown and Gintis, *ibid.*, in the press; (*i*) Brown and Domash, *ibid.*, in the press; (*j*) Brown *et al.*, *ibid.*, in the press; (*k*) Bartlett and Rosen, *ibid.*, 1942, **64**, 543; (*l*) Vernon, *J.*, 1954, 4462; (*m*) Dostrovsky, Hughes, and Ingold, *J.*, 1946, 173.

The steric requirements of the base being constant, the rate of reaction decreases and the energy of activation increases with the increasing steric requirements of the alkyl halide (Fig. 6). Similarly, constancy of the steric requirements of the alkyl halide results in a rate which decreases and an energy of activation which increases with the increasing steric requirements of the pyridine base (Fig. 7). Simultaneous increases in the steric requirements of

FIG. 6. *Effect of steric strain* (S) *on activation energies in the picoline series.*

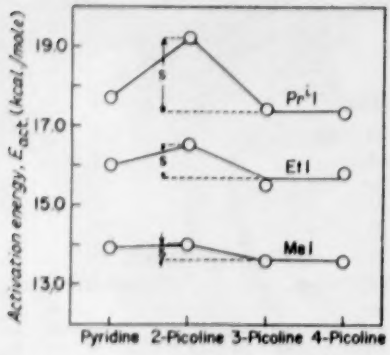
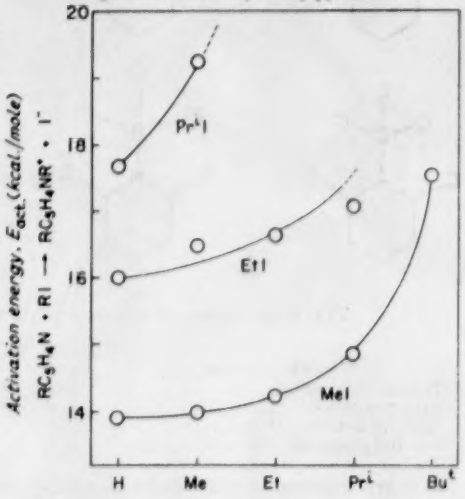



FIG. 7. *Effect of increasing steric strain on activation energies in reactions of 2-alkylpyridines.*




both the alkyl halide and the pyridine base cause cumulative changes in the reaction rates and activation energies. The experimental results are summarized in Table 4.

TABLE 4. *Rate data for the reaction of pyridine bases with alkyl halides in nitrobenzene solution.*

| R in pyridine base R·$C_5H_4N$ | Methyl iodide | | | Ethyl iodide | | | *iso*Propyl iodide | | |
|---|---|---|---|---|---|---|---|---|---|
| | $10^6k_2^{25}$ | $E_{act}$ | log $A$ | $10^6k_2^{25}$ | $E_{act}$ | log $A$ | $10^6k_2^{25}$ | $E_{act}$ | log $A$ |
| H | 343 | 13·9 | 6·72 | 18·3 | 16·0 | 6·98 | 0·941 | 17·7 | 6·93 |
| 2-Me | 162 | 14·0 | 6·46 | 4·27 | 16·5 | 6·70 | 0·0509 | 19·2 | 6·80 |
| 2-Et | 76·4 | 14·2 | 6·31 | 1·95 | 16·6 | 6·49 | — | — | — |
| 2-Pr$^i$ | 24·5 | 14·8 | 6·27 | 0·555 | 17·1 | 6·26 | — | — | — |
| 2-Bu$^t$ | 0·080 | 17·5 | 5·75 | — | — | — | — | — | — |
| 3-Me | 712 | 13·6 | 6·82 | 40·0 | 15·5 | 6·98 | 1·73 | 17·4 | 7·01 |
| 3-Et | 761 | — | — | 41·0 | — | — | 1·81 | — | — |
| 3-Pr$^i$ | 810 | — | — | 40·4 | — | — | 1·68 | — | — |
| 3-Bu$^t$ | 950 | — | — | 43·3 | — | — | 1·56 | — | — |
| 4-Me | 760 | 13·6 | 6·86 | 41·9 | 15·8 | 7·12 | 1·99 | 17·3 | 6·98 |
| 4-Et | 777 | — | — | 42·1 | — | — | 2·01 | — | — |
| 4-Pr$^i$ | 767 | — | — | 42·2 | — | — | 1·98 | — | — |
| 4-Bu$^t$ | 757 | 13·7 | 6·91 | 41·9 | — | — | 2·00 | — | — |

Bimolecular displacement reactions normally show a regular decrease in rate in the order :

$$CH_3X > CH_3{\cdot}CH_2X > (CH_3)_2CHX > (CH_3)_3CX$$

It was originally suggested by Polanyi that this decrease was the result of steric forces.[3g] The results of the study just reviewed appear to be in complete accord with this view. However, adherents of the electronic theory have maintained that the decrease was due to the inductive effect of the alkyl substituents, which by increasing the electron density at the central carbon atom would render the approach of an electronegative reagent increasingly difficult.[3c]

This viewpoint has been used to account for the large increase in activation energy observed in the reactions of triethylamine with methyl and *iso*propyl iodide [$E$(Pr$^i$I) − $E$(MeI) = 6·3 kcal./mole] compared with the smaller increment observed in the corresponding reactions

of pyridine (3·8 kcal./mole). According to the proposed interpretation, the central nitrogen atom of the strong base, triethylamine, would be strongly electronegative and would be greatly affected by the increasing negative (or decreasing positive) charge on the central carbon atom of the *iso*propyl group. On the other hand, the nitrogen atom in the weaker base, pyridine, would be less electronegative and would therefore be much less affected by changes in the polar nature of the central atom of the alkyl group.[34]

The steric interpretation accounts for the phenomena equally well. According to this interpretation, a large increase in activation energy should accompany the reaction of a base with large steric requirements, such as triethylamine, with an alkyl halide with large steric requirements, such as *iso*propyl iodide. A much smaller increase in activation energy should be observed in the corresponding reactions of a base with small steric requirements, such as pyridine.

Quinuclidine offered a critical test of these alternative interpretations. It is a strong base, similar to triethylamine, but with relatively small steric requirements, resembling those of pyridine. On the basis of the polar interpretation, there would be expected a large increase in the activation energy in going from methyl to *iso*propyl iodide. However, the steric theory would predict that its behaviour should more closely resemble that of pyridine with a relatively small increment in the activation energy. The observed increment is small [$E(\mathrm{Pr^i I}) - E(\mathrm{MeI}) = 4{\cdot}1$ kcal./mole], strongly supporting the proposed steric interpretation for the phenomena.

A useful procedure for separating polar and steric effects is to separate the group from the reaction centre by means of an unsaturated system (aryl, vinyl, or ethynyl).[12d] The introduction of methyl groups in the $\gamma$-position of allyl chloride should not alter the steric factor while relaying the polar effect of the methyl groups to the reaction centre. Yet the exchange reaction with chloride ion in acetone shows an *increase* in rate accompanying the presence of the methyl groups.[12g] In the $\alpha$-position the methyl groups sharply decrease the rate of the exchange reaction.[12f]

| | |
|---|---|
| $CH_2{:}CH{\cdot}CH_2Cl$ ..... | 1·00 |
| $CH_2{\cdot}CH{:}CH{\cdot}CH_2Cl$ ..... | 2·52 |
| $(CH_3)_2C{:}CH{\cdot}CH_2Cl$ ..... | 21·3 |
| $CH_2{:}CH{\cdot}C(CH_3)_2Cl$ ..... | 0·043 |

These results argue against the polar interpretation for the decrease in reactivity in the methyl—*tert.*-butyl halide series.

It has now been suggested that approximately half of the decrease with increased branching of the alkyl group is due to steric forces with the remainder due to the operation of polar forces.[12m] It would take us too far afield to consider the argument here. However, it is the Lecturer's view that there do not appear to be any experimental results at present available which require for their explanation a significant contribution from the polar factor in order to account for the observed decrease in reactivity in the alkyl halides under consideration.

It was suggested that steric hindrance effects in displacement reactions are primarily the result of steric strains in the activated complex and should be related to steric strains in molecular addition compounds of similar steric requirements. To test this proposal we have compared the strains in molecular addition compounds with strains present in the activated complexes.

In Fig. 8 there is plotted the heat of reaction of trimethylboron with various pyridine bases against their $pK_a$ values. It will be observed that a reasonably good linear relation is indicated by pyridine and the 3- and 4-alkylpyridines, but that the 2-alkylpyridines deviate greatly. Moreover, the deviations from the line increase regularly from 2-methyl to 2-ethyl to 2-*iso*propyl, with a further sharp increase to 2-*tert.*-butyl. In other words the deviation can be correlated with the steric requirements of the 2-substituent.

With smaller steric requirements of the reference acid (boron trifluoride in Fig. 9 and borine in Fig. 10) the deviations decrease and the points for the 2-alkyl derivatives move toward the line. Finally, with methanesulphonic acid there is realized a complete linear relation for all of the alkylpyridines (Fig. 11).

Figs. 12—15 show similar plots of the heats of reaction of these reference acids with the bases *versus* the activation energies for their reaction with methyl iodide. Again large deviations are observed for trimethylboron, smaller ones for boron trifluoride, while borine yields a linear relation. On the other hand, methanesulphonic acid no longer yields a linear relation, with the 2-alkylpyridines tending to fall on the opposite side of a possible line.

In other words, where the steric requirements of the two reactions being compared are

FIG. 8.

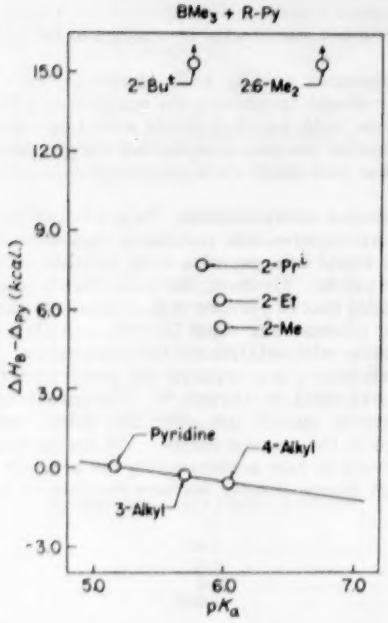



FIG. 9.

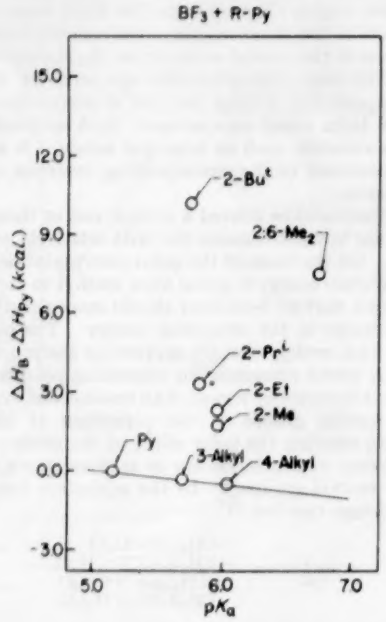



FIG. 10.

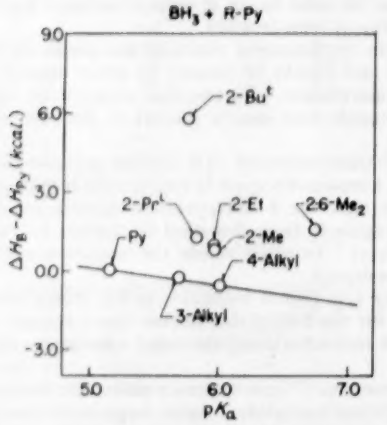
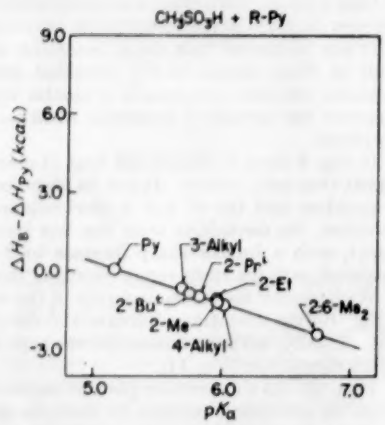



FIG. 11.




FIGS. 8—11. *Relations between the strengths of alkylpyridine bases and their heats of reaction with* (*Fig.* 8) *trimethylboron,* (*Fig.* 9) *boron trifluoride,* (*Fig.* 10) *borine, and* (*Fig.* 11) *methanesulphonic acid.*

FIG. 12.

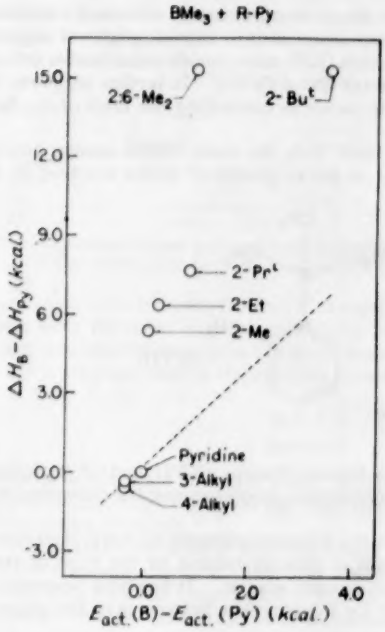



FIG. 13.

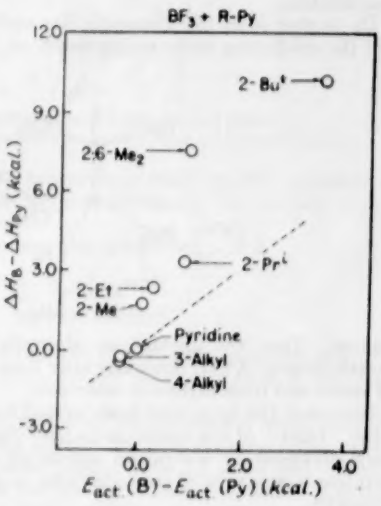



FIG. 14.

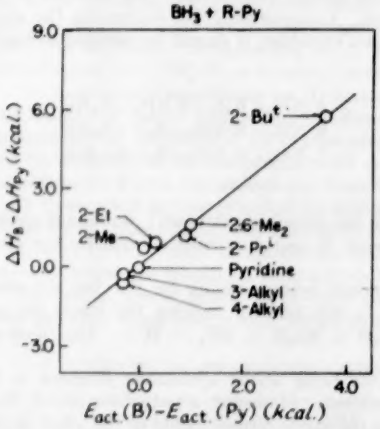
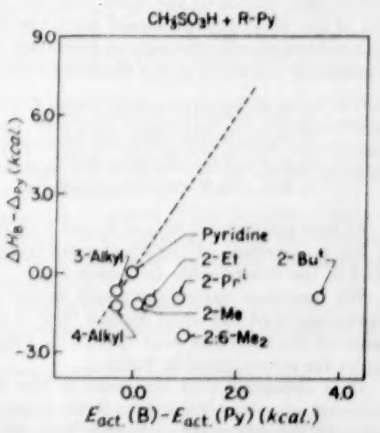



FIG. 15.



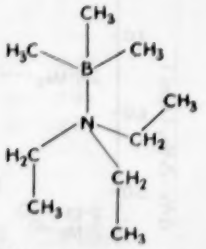

FIGS. 12—15. *Relations between the energies of activation for the reaction of methyl iodide with alkylpyridines and the heats of reaction of these bases with* (*Fig.* 12) *trimethylboron,* (*Fig.* 13) *boron trifluoride,* (*Fig.* 14) *borine, and* (*Fig.* 15) *methanesulphonic acid.*

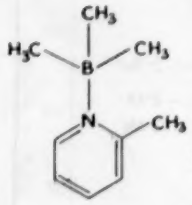
different, the 2-alkylpyridines fail to fit a linear relation. Where the steric requirements of the two reactions are similar ($\Delta H$, $Me{\cdot}SO_3H$ *v.* $pK_a$; $\Delta H$, $\frac{1}{2}(BH_3)_2$ *v.* $\Delta E_{act}$ MeI), satisfactory linear relations are obtained.

This realization of a linear relation between the strain energies in the activated complexes and the strain energies of related molecular addition compounds is encouraging. It suggests that the study of selected molecular addition compounds (XV) may provide considerable information as to the precise nature of the related transition states (XIV). It further supports the conclusion that steric effects constitute an important factor in controlling the rates of displacement reactions.

Up to this point the discussion has concerned itself with the steric effects arising directly from the conflicting steric requirements of the two atoms or groups of atoms involved in the

(XVI)





(XVII)

reaction. Thus the instabilities of triethylamine–trimethylboron (XVI) and of 2-picoline–trimethylboron (XVII) obviously arise from the large strains accompanying the compression of the amine and trimethylboron molecules.

Strains of this type have been termed F-strain since it is due primarily to steric interactions at the " front " of the amine molecule. Such strain is directly related to the type of steric effect envisaged by Kehrmann and Meyer in their pioneer studies. It has been necessary to postulate a new type of strain in order to account for the complete behaviour of the aliphatic amines.[13a]

The $+I$ inductive effect of alkyl groups should result in an increase in the strength of aliphatic amines, $NH_3 < R{\cdot}NH_2 < R_2NH < R_3N$. On the other hand, the steric requirements of the aliphatic groups should tend to reduce the apparent strengths of the amines. In cases where the steric effect outweighs the polar effect, the opposite order should be observed.

By varying the steric requirements of the reference acid, it should be possible to vary the relative importance of the steric and the polar factor within wide limits. An increase in the bulk of the alkyl groups should have the same effect. Consequently, by decreasing the steric strain systematically through an alteration in these two variables, it should be possible to realize a systematic variation in the sequence from 1 to 7 :

1, $R_3N < R_2NH < R{\cdot}NH_2 < NH_3$.
2, $R_3N < R_2NH < NH_3 < R{\cdot}NH_2$.
3, $R_3N < NH_3 < R_2NH < R{\cdot}NH_2$.
4, $NH_3 < R_3N < R_2NH < R{\cdot}NH_2$.
5, $NH_3 < R_3N < R{\cdot}NH_2 < R_2NH$.
6, $NH_3 < R{\cdot}NH_2 < R_3N < R_2NH$.
7, $NH_3 < R{\cdot}NH_2 < R_2NH < R_3N$.

As was pointed out above, 1 and 7 represent the two possible extremes : dominant control by the polar effect of the alkyl substituents (sequence 7) and dominant control by the steric effect of the substituents (sequence 1).

We therefore varied the steric factor in the amines systematically by varying the steric requirements of the alkyl groups ($Bu^t > Pr^i > Et > Me$) and by varying the steric requirements of the reference acid ($Bu^t_3B > Pr^i_3B > Et_3B > Me_3B > BF_3 > H^+$). The observed results are summarized in Table 5.

It is apparent that decreases in the steric factor bring about systematic changes in the observed sequences from 1 to 2, to 3, etc., as predicted. However, a reduction of the steric factor to the minimum realizable, both by reducing the steric requirements of the alkyl groups

[13] B-Strain : (*a*) Brown, Bartholomay, jun., and Taylor, *J. Amer. Chem. Soc.*, 1944, **66**, 435; (*b*) Spitzer and Pitzer, *ibid.*, 1948, **70**, 1261; (*c*) Lawton, Thesis, Purdue, 1952; (*d*) Sujishi, Thesis, Purdue, 1949; (*e*) Fletcher, Thesis, Purdue, 1952.

in the amine to methyl and by reducing the steric requirements of the reference acid to the proton, fails to achieve the theoretical limiting sequence for the complete absence of the steric factor. Instead the sequences appears to be approaching 5 as a limit. As was pointed out earlier, the steric requirements of the proton are exceedingly small. No steric effect towards the proton is exhibited by 2 : 6-lutidine in spite of large steric requirements which prevent it from

TABLE 5. *Observed sequences for alkylamines.*[13c]

| Amines | Reference acids: $Bu^t_3B$ | $Pr^i_3B$ | $Et_3B$ | $Me_3B$ | $BF_3$ | $H^+$ |
|---|---|---|---|---|---|---|
| $Bu^t$ [a] | 1 | 1 | 1 | 1 | 1 or 2 | — |
| $Pr^i$ [b] | 1 | 1 or 2 | 1 or 2 | 2 | 2 | 5 |
| Et | 1 or 2 | 2 | 2 | 3 | 3 | 5 |
| Me | 2 | 2 or 3 | 3 | 5 | 5 | 5 |

[a] Complete order deduced from behaviour of ammonia and mono-*tert.*-butylamine.
[b] Complete order deduced from behaviour of ammonia and mono- and di-*iso*propylamine.

combining with trimethylboron. Consequently, we appear to be forced to the conclusion that even with reference acids of essentially negligible steric requirements the limiting sequence contains a contribution from the steric factor.

It is proposed that in the reaction of tertiary amines with a reference acid Z

$$\underset{\text{(strained)}}{R_3N} + Z \rightleftharpoons \underset{\text{(more strained)}}{R_3N{:}Z}$$

there is an increase in steric strain which is independent of the steric requirements of the reference acid Z. This strain has been termed B-strain since it arises primarily at the "back" of the amine molecule.

Calculations have been reported which suggest that such strains are too small to account for the observed effects.[13b] We have also attempted to calculate B-strain by utilizing spectroscopic data and the Urey–Bradley force field. Values ranging from 1·5 to 10 kcal./mole were obtained.[13e] We also carried out a calculation of the energy required to distort a molecule such as $NA_3$ (considered to be homomorphic with $NMe_3$) utilizing the potential function for argon. We again obtained an answer which points to the presence of considerable strain.[13e] Although these theoretical approaches support the presence of B-strain in trimethylamine and related molecules, the approximations which must be utilized are such as to give one much more confidence in the experimental approach to such problems.

If the strain in trimethylamine is the result of the crowding of the three methyl groups around the small nitrogen atom, the effect should be greatly reduced or absent in the corresponding phosphorus derivatives with their much larger central atom. Indeed, the methylphosphines yield the sequence corresponding to 7, $PH_3 < MePH_2 < Me_2PH < Me_3P$, with $H^+$,[13d] with $BMe_3$,[13d] and with $BF_3$ [13e] as reference acids.

We now have observations on (1) ionization in aqueous solution, (2) dissociation of addition compounds of boron trifluoride, (3) dissociation of addition compounds of trimethylboron, (4) volatility of 'onium salts, (5) vapour pressures in systems with acetic and trifluoroacetic acids, and (6) dissociation of compounds with *n*-dodecanesulphonic acid.[13d] In all cases, the results indicate order 5 with the amines and order 7 with the phosphines.

If three alkyl groups attached to nitrogen constitute a centre of strain, it follows from the similarity in atomic dimension that three alkyl groups attached to carbon should also constitute a centre of strain. Such strain should manifest itself in an increased reactivity of molecules in reactions where the transition state affords a decrease in strain.

Classically, steric effects have been considered synonymous with steric hindrance. The proposal that steric effects may serve to assist a reaction represents a sharp departure from tradition.[14a]

The validity of the proposal was examined in the solvolysis of the tertiary alkyl chlorides.

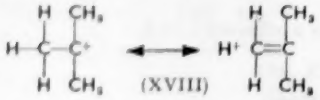
[14] Steric assistance in unimolecular reactions: (*a*) Brown, *Science*, 1946, **103**, 385; (*b*) Brown and Fletcher, *J. Amer. Chem. Soc.*, 1949, **71**, 1845; (*c*) *idem*, *ibid.*, 1950, **72**, 1223; (*d*) Brown and Stern, *ibid.*, p. 5068; (*e*) Brown and Fletcher, *ibid.*, 1951, **73**, 1317; (*f*) Brown and Kornblum, *ibid.*, 1954, **76**, 4510; (*g*) Burawoy and Spinner, *J.*, 1954, 3752; (*h*) Bartlett and Swain, *J. Amer. Chem. Soc.*, 1955, **77**, 2801; (*i*) F. Brown, Davies, Dostrovsky, Evans, and Hughes, *Nature*, 1951, **167**, 987; (*j*) Hughes, Ingold, and Shiner, jun., *J.*, 1953, 3827; (*k*) Winstein and Trifan, *J. Amer. Chem. Soc.*, 1949, **71**, 2953; 1952, **74**, 1147, 1154; (*l*) Bartlett, *J. Chem. Educ.*, 1953, **30**, 22.

It was considered that strain present in the initial tetrahedral molecule should be greatly reduced in the formation of the transition state leading to the planar carbonium ion :

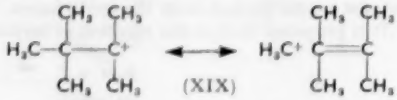
$$\underset{\text{tetrahedral (strained)}}{R_3C{\cdot}Cl} + H_2O \xrightarrow{k_1} \underset{\text{planar (less strained)}}{R_3C^+} + Cl^-$$

If steric assistance were indeed a factor in this reaction, the introduction of one or more bulky R groups into the tertiary halide should be accompanied by an enhanced rate of solvolysis. The replacement of one of the methyl groups in *tert.*-butyl chloride by a *tert.*-butyl group causes an increase in the first-order rate constant (80% ethanol) from 0·033 hr.$^{-1}$ to 0·040. A *tert.*-pentyl (1 : 1-dimethylpropyl) group causes the rate to rise further to 0·188 hr.$^{-1}$. These increases are in the direction predicted by the theory.[14b] However, it is necessary to enquire whether they might arise from other causes.

A dominant factor in the rapid solvolysis of *tert.*-butyl chloride must be the stabilization of the carbonium ion through hyperconjugation (XVIII). The replacement of the methyl group by the *tert.*-butyl (as in XIX) or the *tert.*-pentyl group would be expected to result in a decrease in the hyperconjugative stabilization and, consequently, a decrease in the observed rate of solvolysis.

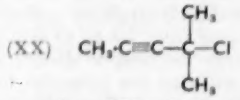


(XVIII)

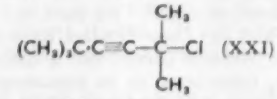


(XIX)

For the two compounds (XX, XXI), the steric factor at the reaction centre has been maintained essentially constant, so the solvolysis rates should reflect the respective electrical contributions of the methyl and *tert.*-butyl groups. In these compounds the *tert.*-butyl derivative exhibits much the slower rate of solvolysis (80% acetone).[14g]

(XX) 

$k_1 = 5{\cdot}13 \times 10^{-4}$ sec.$^{-1}$

 (XXI)

$k_1 = 2{\cdot}80 \times 10^{-4}$ sec.$^{-1}$

Consideration of other contributing factors, such as the ease of solvation of the incipient carbonium ion and the ease of electrophilic attack of the solvent on the halogen likewise leads to the prediction that the bulky *tert.*-butyl and *tert.*-pentyl substituents should decrease the solvolysis rate. The observed increases may therefore be considered to support the postulated rôle of steric strain in assisting the ionization.

Similarily the replacement of one of the methyl groups in *tert.*-butyl chloride by a *neo*pentyl group, giving a homomorph of di-*tert.*-butylmethane, results in a 21-fold increase in the reaction rate. Two *neo*pentyl groups raise the rate to 580 times that of *tert.*-butyl chloride.[10c]

Even larger effects have been realized by Bartlett and Swain [14h] and by F. Brown *et al.* [14f] in the solvolysis of *tert.*-alkyl derivatives containing a number of bulky substituents.

Bridging by neighbouring carbon has been utilized to account for the enhanced solvolysis rates exhibited by certain bicyclic systems.[14i] The possibility that similar phenomena may be responsible for the enhanced rates of ionization in the highly branched tertiary halides has been considered.[14j] However, all attempts to establish the importance of such bridging in compounds of the type under consideration here have yielded negative results.[14f] As a result there does not appear to be any need to bring in this additional factor in accounting for the enhanced ionization rates of highly branched tertiary derivatives.

The carbonium ion, once formed, can react by three different paths. It can react with the solvent to regenerate the original tetrahedral system ($k_s$), it can lose a proton, forming an olefin ($k_E$), or it can undergo rearrangement to form a new carbonium ion. It is reasonable that a carbonium ion containing bulky substituents should react somewhat more slowly with the solvent. Consequently such an ion should exhibit an enhanced tendency to undergo elimination and rearrangement. Typical data are summarized in Table 6.[14c]

TABLE 6. *Elimination in the solvolysis of tertiary chlorides at 25° in 80% ethanol.*

| Carbonium ion ..... | $Me_3C^+$ | $Bu^tMe_2C^+$ | $Et_3C^+$ | $Bu^tEt_2C^+$ | $Me{\cdot}CH_2{\cdot}Me_2C^+$ | $Bu^t{\cdot}CH_2{\cdot}Me_2C^+$ |
|---|---|---|---|---|---|---|
| Yield (%) of olefin... | 16 | 61 | 40 | 90 | 34 | 65 |

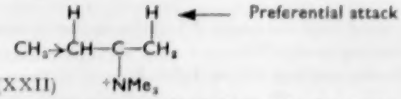
Alternative interpretations of these results have been presented.[14j] However, a considerable body of additional results now supports the original conclusions. Consequently, there appears to be no need to revise the interpretation as presented above.

It is observed that *tert.*-butyldiethylcarbinol (3-ethyl-2 : 2-dimethylpentan-3-ol) rearranges on reaction with hydrogen chloride at 0°,[14b] whereas the closely related compound *tert.*-butylethylmethylcarbinol (2 : 2 : 3-trimethylpentan-3-ol) reacts without rearrangement.[14f] It was pointed out that the *tert.*-butyldiethylcarbonium ion will have both faces of the ion blocked by the two ethyl groups so that the rate of substitution will be reduced, whereas the *tert.*-butylethylmethylcarbonium ion is relatively unhindered and a much faster rate of substitution appears likely for it. The rearrangement observed in the reaction of *tert.*-butyldiethylcarbinol is therefore attributed to the relatively low rate of substitution, providing a better opportunity for the ion to undergo structural change.

The concept of steric assistance provides a simple, unifying explanation for the effect of structure upon the ionization, elimination, and rearrangement reactions of highly branched carbonium ions.

The effect of structure upon the rate, yield, and directive effects of olefin formation in the elimination reaction had previously been given careful consideration by Hughes and Ingold and their co-workers.[15k] They concluded that hyperconjugative and polar factors were of importance and attributed all of the observed phenomena to the operation of these factors.

For example, in all unimolecular eliminations and in bimolecular eliminations of alkyl halides the major product is the most alkylated of the possible olefins (Saytzeff rule). This was attributed to hyperconjugative stabilization of the incipient double bond in the transition state by the alkyl groups. On the other hand, bimolecular elimination of 'onium salts yields preferentially the least alkylated olefin (Hofmann rule). It was suggested that under the influence of the positive charge in the 'onium group there is a flow of electrons from the β-carbon atoms. This is resisted by the inductive effects of alkyl groups, resulting in preferential attack by the base at the least heavily alkylated β-carbon atom (XXII).




(XXII)

On the other hand, Schramm has suggested that it is the steric requirements and not the charge of the 'onium group which is responsible for the observed direction of elimination.[15l]

Our studies of the solvolysis of *tert.*-alkyl chlorides had led us to the conclusion that steric effects were a factor in the yield of olefin in these unimolecular reactions. It appeared desirable to examine both unimolecular and bimolecular eliminations in order to test the importance of the steric factor in these reactions.

The solvolysis of a series of closely related tertiary bromides, R·$CH_2$·$CMe_2$Br, revealed that the yield and the nature of the product varied in a regular manner with the increasing steric requirements of the group R (Table 7). Moreover, in the last member of the series, R = $Bu^t$,

TABLE 7. *Experimental data on the yield and composition of olefins formed in solvolysis.*

| Compound * | R | Yield (%) | Composition (%) 1- | *trans*-2 | *cis*-2 |
|---|---|---|---|---|---|
| $Me_2EtCBr$ [a] | Me | 27 [15b] | 20 | 80 | |
| $Me_2Pr^nCBr$ [a] | Et | 32·5 [15b] | 29 | 71 | |
| $Me_2Bu^iCBr$ [a] | $Pr^i$ | 46 [15b] | 41 | 59 | |
| $Bu^t$·$CH_2$·$CMe_2Br$ [a] | $Bu^t$ | 57 [15b] | 82 | 18 | |
| $MePr^nCHBs$ [b] | Et | 31·5 [15c] | 15·7 | 48·8 | 35·5 |
| $MeBu^iCHBs$ [b] | $Pr^i$ | 46·5 [15c] | 19·7 | 53·0 | 27·3 |
| $Bu^t$·$CH_2$·$CHMeBs$ [b] | $Bu^t$ | 68 [15c] | 24·3 | 74·8 | 0·9 |

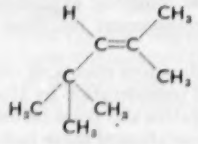
* Bs = *p*-Br·$C_6H_4$·$SO_2$·O. [a] 85% $Bu^nO$·$CH_2$·$CH_2$·OH at 25°. [b] Anhyd. AcOH at 70°.

the product consisted of 82% of the 1-olefin, a clear case of elimination in accordance with the Hofmann rule.[15a, b]

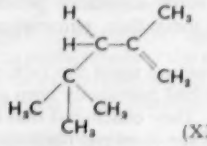
[15] Steric effects in elimination reactions: (*a*) Brown and Moritani, *J. Amer. Chem. Soc.*, **1955**, **77**, 3607; (*b*) Brown and Nakagawa, *ibid.*, p. 3610; (*c*) *idem*, *ibid.*, p. 3614; (*d*) Brown and Okamoto, *ibid.*, p. 3619; (*e*) Brown and Moritani, *ibid.*, p. 3623; (*f*) Brown, Moritani, and Nakagawa, *ibid.*, in the press; (*g*) Brown, Moritani, and Okamoto, *ibid.*, in the press; (*h*) Brown and Nakagawa, *ibid.*, in the press; (*i*) Brown and Wheeler, *ibid.*, in the press; (*j*) Brown and Moritani, *ibid.*, in the press; (*k*) Dhar, Hughes, Ingold, Mandour, and Maw, *J.*, 1948, 2093; (*l*) Schramm, *Science*, 1950, **112**, 367.

It was suggested that in the simpler members of this series the predominant formation of the 2-olefin is due to its greater stabilization by hyperconjugation, as proposed by Hughes and Ingold. However, the 2-olefin contains the alkyl group R in a position *cis* to one of the methyl groups. As the steric requirements of R increase, the steric interactions between R and the methyl group likewise increase. With R = *tert.*-butyl (XXIII), the strains become sufficiently large to overcome the effect of hyperconjugative stabilization and the 1-olefin becomes the preferred product (XXIV).

This conclusion is confirmed by the results obtained in the solvolysis of the corresponding secondary derivative, $(CH_3)_3C{\cdot}CH_2{\cdot}CH(Bs){\cdot}CH_3$ (Bs = *p*-bromophenylsulphonyloxy). Here the solvolysis product is predominantly the *trans*-2-olefin. The molecule retains the electronic stabilization of the 2-olefin while avoiding the large strains of the *cis-tert.*-butyl-methyl relation in 2 : 4 : 4-trimethylpent-2-ene (XXIII). The experimental results are summarized in Table 7.



(XXIII)



(XXIV)

In treating the series of tertiary bromides $R{\cdot}CH_2{\cdot}CMe_2Br$ with potassium ethoxide (*E*2) we also observed a regular increase in the yield of 1-olefin in the product with the increasing steric requirements of R. By increasing the steric requirements of the alkoxide base (EtOK < $Bu^tOK$ < $EtMe_2C{\cdot}OK$ < $Et_3C{\cdot}OK$) [15g] or the pyridine base (pyridine < 2-picoline < 2 : 6-lutidine) [15h] there was again observed with each of the alkyl halides a regular increase in the amount of the 1-olefin formed in the reaction.

Indeed, in the case of simple alkyl halides, such as *tert.*-pentyl, it proved possible to shift the elimination from typically Saytzeff to typically Hofmann type merely by varying the steric requirements of the base.

Finally, it was demonstrated that the yield of 1-olefin increases regularly with the increasing steric requirements of the leaving group.[15f,j]

The experimental results are summarized in Table 8.

TABLE 8. *Percentage of* 1*-olefin formed in the bimolecular elimination reactions of various* 2*-substituted derivatives* RX.[15]

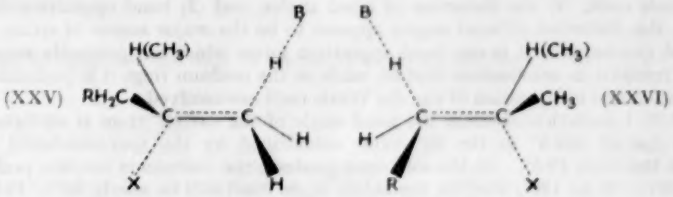
| R | X * | Pyridine | 2-Picoline | 2 : 6-Lutidine | $EtO^-$ | $Bu^tO^-$ | $EtMe_2C{\cdot}O$ | $Et_3C{\cdot}O^-$ |
|---|---|---|---|---|---|---|---|---|
| MeEtCH ..... | Br | — | — | — | 19 [15k] | 53 | — | — |
| | OTs | — | — | — | — | 62 | — | — |
| | $SMe_2^+$ | — | — | — | 70 [15k] | — | — | — |
| $MePr^nCH$ ... | Br | — | — | — | 31 | 66 | — | — |
| | I | — | — | — | 30 | — | — | — |
| | OTs | — | — | — | 47 | 73 | — | — |
| | $SMe_2^+$ | — | — | — | 87 | — | — | — |
| | $SO_2Me$ | — | — | — | 89 | — | — | — |
| | $NMe_3^+$ | — | — | — | 98 | — | — | — |
| $Me_2Pr^iCH$ ... | Br | 10 | 18 | 37·5 | 21 | 73 | 81 | 92 |
| | OTs | 43 | — | — | — | — | — | — |
| $Me_2EtC$ ..... | Cl | — | — | — | — | 72 | — | — |
| | Br | 25 | 30 | 44·5 | 30 | 72·5 | 77·5 | 88·5 |
| | OTs | 37 | — | — | — | — | — | — |
| | $SMe_2^+$ | — | — | — | 86 [15k] | — | — | — |
| | $NMe_3^+$ | — | — | 92·5 | 93 | — | — | — |
| $Me_2Pr^nC$ ..... | Br | 32 | 39 | 48 | 50 | — | — | — |
| $Me_2Bu^tC$ ..... | Br | 44 | 52 | 58 | 54 | — | — | — |
| $Bu^tCH_2{\cdot}CMe_2$ | Cl | — | — | — | 86 | — | — | — |
| | Br | 70 | 74 | 82 | 86 | 98 | — | 97 |
| | OTs | 75 | — | — | — | — | — | — |
| | $NMe_2^+$ | 88 | 95 | 99 | 100 | — | — | — |

* In order of increasing steric requirements : $Br < OTs < SMe_2^+ < SO_2Me < NMe_2^+$.
Ts = *p*-$Me{\cdot}C_6H_4{\cdot}SO_2$.

It is suggested that the bimolecular elimination proceeds through the transition states (XXV, XXVI).

In the absence of steric strain the reaction will proceed preferentially through the transition state (XXVI) to give the more alkylated of the two possible olefins (Saytzeff rule). With increasing steric requirements of either R or X (or both), steric strain will decrease the stability

of this transition state. The alternative state (XXV) will be much less affected. Consequently there will occur an increased tendency for the reaction to proceed through transition state (XXV) to form the thermodynamically less stable 1-olefin. An increase in the steric requirements of the base, B, should also result in far greater strain in transition state (XXVI), where a secondary hydrogen must be removed, than in transition state (XXV), where a primary hydrogen is eliminated.




Transition state leading to 1-olefin

Transition state leading to 2-olefin

This interpretation accounts in a simple manner for the observation that a shift from the more alkylated to the less alkylated olefin can be achieved by (1) an increase in the steric requirements of the alkyl groups on the incipient double bond, (2) an increase in the steric requirements of the leaving group, and (3) an increase in the steric requirements of the attacking base.

For simplicity the present discussion has been restricted to the reactions of 2-substituted derivatives, whose elimination reactions yield 1- or 2-olefins. Generalization to more complex examples appears to offer no difficulty.

The available data support the interpretation that elimination in accordance with the Saytzeff rule represents control by the hyperconjugative factor, whereas elimination in accordance with the Hofmann rule represents control by the steric factor.

Steric effects also appear to be an important factor in the chemical behaviour of simple ring systems. Thus, in the small rings (3- and 4-membered) the *cyclo*alkyl halides, with a few exceptions, are unusually inert in typical substitution reactions.[16e] On the other hand, *cyclo*butanone is far more reactive than open-chain ketones.[16f]

Similar phenomena are observed in the common rings (5-, 6-, and 7-membered). Here *cyclo*pentyl and *cyclo*heptyl derivatives are quite reactive in substitution reactions, whereas the ketones are relatively inert. On the other hand, *cyclo*hexanone is very reactive and *cyclo*hexyl derivatives are inert.[16b, d]

The behaviour of the medium rings (8- to 12-membered) has been the subject of a thorough discussion in an earlier Centenary Lecture.[16g] Here also it is observed that the ketones and tetrahedral ring derivatives exhibit opposite reactivities. Thus the ring ketones in this range are highly inert in the usual ketone reactions, whereas the *cyclo*alkyl toluene-*p*-sulphonates [16c] or 1-chloro-1-methyl*cyclo*alkanes [16c] exhibit unusually high reactivities in solvolysis.

Thus the change in co-ordination number of a ring atom may be either favoured or not, depending upon the number of ring atoms. However, from the available data it appears to be a fairly general characteristic of these ring systems that in cases where the ring size favours a reaction which proceeds *via* a decrease in the co-ordination number, *e.g.*,

the opposite type of reaction

proceeds with relative difficulty.

[16] Chemistry of ring compounds: (*a*) Brown and Gerstein, *J. Amer. Chem. Soc.*, 1950, **72**, 2926; (*b*) Brown, Fletcher, and Johannesen, *ibid.*, 1951, **73**, 212; (*c*) Brown and Borkowski, *ibid.*, 1952, **74**, 1894; (*d*) Brown, Brewster, and Shechter, *ibid.*, 1954, **76**, 467; (*e*) Brown and Ham, *ibid.*, in the press; (*f*) Brown and Ichikawa, *ibid.*, in the press; (*g*) Prelog, *J.*, 1950, 420; (*h*) Bartlett, *Bull. Soc. chim. France*, 1951, c, 100; (*i*) Roberts and Chambers, *J. Amer. Chem. Soc.*, 1951, **73**, 5034; (*j*) Fierens and Verschelden, *Bull. Soc. chim. belges*, 1952, **61**, 427, 609; (*k*) Van Straten, Nicholls, and Winckler, *Canad. J. Res.*, 1951, **29**, 372; (*l*) Trotman-Dickenson and Steacie, *J. Chem. Phys.*, 1951, **19**, 329; (*m*) Overberger, Biletch, Finestone, Lilker, and Herbert, *J. Amer. Chem. Soc.*, 1953, **75**, 2078.

It has been proposed that this phenomenon is the result of changes in internal strain in these ring systems. Where a decrease in the co-ordination number of the ring atom (from 4 to 3, or $sp^3$ to $sp^2$) results in a decrease in internal strain, the reaction will be favoured; consequently, the opposite change (from 3 to 4, or from $sp^2$ to $sp^3$) will cause an increase in strain and the reaction will be hindered.[16b]

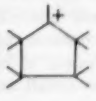
At the present time we recognize three sources of steric strains: (1) the compression of van der Waals radii, (2) the distortion of bond angles, and (3) bond opposition forces. For small rings, the distortion of bond angles appears to be the major source of strain. In *cyclo*pentane and *cyclo*heptane it is the bond opposition forces which are primarily responsible for the strains revealed in combustion studies, while in the medium rings it is probable that both bond opposition and compression of van der Waals radii are involved.

In 1-chloro-1-methyl*cyclo*butane the bond angle of the carbon atom is distorted from its preferred value of 109·5° to the 90° value established by the four-membered ring. The distortion is therefore 19·5°. In the solvolysis product, the carbonium ion, the preferred bond angle is believed to be 120°, whereas the actual angle must still be nearly 90°. This results in an increase of the distortion to 30°. It is suggested that such a reaction will be accompanied by an increase in angle strain and will therefore be resisted.

The identical arguments in opposite order apply to *cyclo*butanone. The reactions of this ketone should be accompanied by a decrease in angle strain and such reactions should be relatively fast.

In *cyclo*pentane angle strain is presumably small. Bond opposition forces are believed responsible for the large strain which is present. In the idealized planar model (XXVII) there will be 10 bond oppositions and approximately 10 kcal./mole strain. The removal of one atom will result in a decrease in the bond oppositions to 6 and a decrease in the estimated strain to 6 kcal./mole (XXVIII).

10 bond oppositions
10 kcal. strain
(XXVII)



6 bond oppositions
6 kcal. strain
(XXVIII)

In the actual molecule the strains are believed to be reduced by some 4 kcal. by some distortion of the bond angles and the assumption of a non-planar arrangement. Presumably similar distortion will occur in the carbonium ion so that the loss of one bond will still be accompanied by a considerable reduction in the internal strains.

*cyclo*Heptane is also strained because of bond opposition forces. Loss of one bond will also be accompanied by a decrease in internal strain.

*cyclo*Hexane, on the other hand, has all of its atoms in an ideally staggered arrangement. Loss of one atom results in a loss of this ideal arrangement and in the introduction of two bond oppositions. Consequently a change in co-ordination from 4 to 3 will be accompanied by an increase in internal strain and the reaction will be resisted.

Applying this interpretation to the corresponding ketones leads to the conclusion that *cyclo*hexanone should be relatively reactive, and *cyclo*pentanone and *cyclo*heptanone relatively inert.[16h]

In the middle rings the effects appear to be considerably larger with a maximum or minimum, depending upon the reaction type, observed in rings of 8- to 10-members.[16g, c] Because of the magnitude of the effect it is suggested that van der Waals deviations as well as bond oppositions are involved. Here again the strain will be relieved by loss of a bond. Consequently, reactions which proceed with a decrease in co-ordination number will be favoured; those which proceed with an increase in co-ordination number will be hindered.

The rate-determining step in free-radical reactions is usually the removal of a ring atom to form the free radical (change in co-ordination number from 4 to 3). The arguments used above should then apply to free radical reactions.[16f, m]

Bimolecular displacement reactions are more difficult. The pentaco-ordinate transition state will be similar to that involved in ionization reactions in having a 120° angle between the groups not involved in the displacement. Consequently, the effects on angle strain will be

similar to that observed in ionization reactions. However, where bond opposition forces are involved the additional bond in the transition state may represent an additional factor in the opposite direction. Unfortunately, the experimental results for this type of reaction are relatively few. Those available suggest that the effects of ring size are similar to those observed in ionization reactions, but with smaller effects in the rings of 7 and 8 members than would be predicted from the results of ionization reactions. The available experimental results are summarized in Table 9.

TABLE 9. *Experimental data on the reactivity of ring compounds :* $[CH_2]_{n-1}$ C<

| | $S_N1$ | | Ketone | | $S_N2$ | | | Free radical | |
|---|---|---|---|---|---|---|---|---|---|
| | Toluene-*p*-sulphonate | *tert.*-Chloride | Cyanohydrin formation | Borohydride reduction | RI + I* | RBr + $I^-$ | | Decomp. of azonitriles | RH + $CH_3$ |
| *n* | $k_1$ (70°) [a] | $k_1$ (25°) [b] | $K_{eq}$ (22—23°) [c] | $k_2$ (0°) [d] | $k_2$ (25°) [e] | $k_2$ (25°) [f] | $k_2$ [g] | $k_1$ (80°) [h] | $k_2$ (182°) |
| 3 | — | — | — | — | — | no reaction (100°) | | — | 0·11 |
| 4 | 11·3 | 0·211 | — | 1·64 | — | 0·760 | 1·42 (90°) | 0·0275 | 0·76 |
| 5 | 14·0 | 124 | 0·048 | 0·0435 | — | 161 | 5·68 (60°) | 11·5 | 1·3 |
| 6 | 1·00 | 1·00 | 1·00 | 1·00 | 1·00 | 1·00 | 1·00 (90°) | 1·00 | 1·00 |
| 7 | 25·4 | 108 | 0·0077 | 0·00634 | 140 | 98·5 | — | 194 | — |
| 8 | 191 | 286 | 0·0012 | 0·000485 | 33 | 19·1 | — | 1320 | — |
| 9 | 173 | 43·9 | 0·00059 | 0·000196 | — | — | — | — | — |
| 10 | — | 17·7 | very small | 0·000082 | — | — | — | 293 | — |
| 11 | 49·1 | 12·0 | 0·00089 | 0·000146 | | | | | |
| 12 | 3·25 | — | 0·0032 | 0·00113 | | | | | |
| 13 | 3·51 | 2·85 | 0·0039 | 0·00120 | | | | | |
| 14 | 1·32 | — | 0·017 | — | — | | | | |
| 15 | 2·19 | 1·81 | 0·0091 | 0·00261 | | | | | |
| 16 | — | — | 0·011 | — | | | | | |
| 17 | 2·18 | 1·90 | 0·0083 | 0·00368 | | | | | |
| 18 | — | — | 0·010 | — | | | | | |
| 19 | — | — | 0·010 | — | | | | | |
| 20 | — | — | 0·014 | — | | | | | |

[a] Acetolysis in glacial acetic acid. First-order constant of *cyclo*hexyl ester at 70°, $k_1 = 2{\cdot}37 \times 10^{-4}$ sec.$^{-1}$. Ref. 16*e*. [b] Solvolysis of the 1-chloro-1-methyl*cyclo*alkanes in 80% ethanol. First-order constant of 1-chloro-1-methyl*cyclo*hexane at 25°, $k_1 = 0{\cdot}0106$ hr.$^{-1}$. Ref. 16*c*. [c] Equilibrium of the cyanohydrin formation in 96% ethanol. Calculated from the dissociation constants of cyanohydrins by Prelog and Kobelt. Dissociation constant of *cyclo*hexanone cyanohydrin at 22—23° : $K_{eq} = [C_6H_{10}(OH)\cdot CN]/[HCN][C_6H_{10}O] = 0{\cdot}1 \times 10^{-3}$. Ref. 16*g*. [d] Reduction of ketones with sodium borohydride in propan-2-ol. Second-order constants of *cyclo*hexanone at 0° : $k_2 = 161 \times 10^{-4}$ l. mole$^{-1}$ sec.$^{-1}$. Ref. 16*f*. [e] Second-order constant of the reaction RI + NaI* ⟶ RI* + NaI in ethanol. For *cyclo*hexyl iodide at 25°, $k_2 = 0{\cdot}0084 \times 10^{-3}$ c.c. mole$^{-1}$ sec.$^{-1}$. Ref. 16*h*. [f] Second-order constant of the reaction, RBr + LiI ⟶ RI + LiBr, in acetone. For *cyclo*hexyl bromide at 25°, $k_2 = 0{\cdot}0129 \times 10^{-3}$ l. mole$^{-1}$ sec.$^{-1}$. Ref. 16*j*. [g] Second-order constant of the reaction, RBr + KI ⟶ RI + KBr, in acetone. For *cyclo*hexyl bromide at 90°, $k_2 = 0{\cdot}0077$ l. mole$^{-1}$ min.$^{-1}$. Ref. 16*i*. [h] Decomp. of azonitriles in toluene, NC·R·N:N·R·CN ⟶ 2NC·R· + $N_2$. First-order constant of the *cyclo*hexyl derivative at 80°, $k_1 = 0{\cdot}063 \times 10^{-4}$ sec.$^{-1}$. Ref. 16*m*. [i] Relative rates of the abstraction, by ·$CH_3$, of hydrogen atoms from *cyclo*alkanes, per active hydrogen. Ref. 16*l*.

It was pointed out that ring compounds contain strains arising from three different types of steric interaction. A change in co-ordination of a ring atom will greatly alter the magnitudes of each of these sources of strain. Ideally it would be desirable to be able to estimate each of these strains independently and to discuss each reaction in terms of its effects on each of these strains. Unfortunately, this is not possible at present. We can, however, estimate from both equilibrium and kinetic data the net change in internal strain accompanying the change in co-ordination of the ring atom. The term I-strain has been proposed as a convenient term for the sum total of the internal strains in ring systems arising from van der Waals compressions, bond-angles distortion, and bond opposition forces.

The results support the conclusion that I-strain is a very important factor in the reactions of ring compounds. It should, however, be understood that it cannot be the only factor. Obviously the use of a very bulky reagent will introduce F-strain effects which will vary with the ring size, and if sufficiently large might alter the order considerably from that predicted on the basis of I-strain. Similarly, if the change in co-ordination number of the ring atom is not involved in the rate-determining step, the I-strain theory cannot be applied to predict the effect

of ring size on chemical reactivity. In spite of these limitations, the theory has proved very useful in systematizing the effects of ring size on chemical behaviour.

In conclusion I wish to point out that the Centenary Lecturer is requested to review the work of his research group in a given area. Needless to say the work I have presented has been made possible only by the co-operation of my very capable graduate students and associates. Moreover, in reviewing our own work, it has not been possible to pay adequate tribute to the numerous other workers who have, since 1940, completely altered the then prevailing opinion with regard to the importance of steric effects as a factor in chemical behaviour. In an effort to provide a proper perspective, the following outline of major developments in arriving at the present position is presented.

*Outline of major developments in the study of steric effects.*

I. Steric Forces.
- *A*. Atomic compressions : F. Kehrmann, 1889.
- *B*. Angle deformation : A. Baeyer, 1885.
- *C*. Bond oppositions : K. S. Pitzer, 1937; V. Shomaker, G. B. Kistiakowsky, 1939.

II. Steric Concepts.
- *A*. Steric hindrance : F. Kehrmann, 1889; V. Meyer, 1894.
- *B*. Steric assistance : H. C. Brown, 1946.

III. Application to Chemical Phenomena.
- *A*. Steric hindrance in aromatic substitution : A. F. Holleman, 1924; R. J. W. Le Fèvre, 1933.
- *B*. Hindered rotation in the diaryls : E. E. Turner and R. J. W. Le Fèvre, W. Mills, F. Bell, and J. Kenner, 1926.
- *C*. Steric hindrance in alkyl halides : M. Polanyi, 1932.
- *D*. Steric hindrance in *neo*pentyl derivatives : P. D. Bartlett, 1942; C. K. Ingold, 1946.
- *E*. Steric assistance in reactions of highly branched compounds : H. C. Brown, 1949.
- *F*. Angle strain in reactions of small rings : H. C. Brown, 1950.
- *G*. Bond oppositions in the chemistry of common rings : O. Hassell, 1942; K. S. Pitzer, 1945; P. D. Bartlett, 1951; H. C. Brown, 1951.
- *H*. Bond oppositions in the reactions of medium rings : V. Prelog, 1950.
- *I*. Bond oppositions in the reactions of steroids : D. H. R. Barton, 1950.
- *J*. Strain in the direction of elimination reactions : C. H. Schramm, 1950; H. C. Brown, 1953.

IV. Quantitative Approaches to Steric Effects.
- *A*. Activation energies in the racemization of diaryls : R. Adams, 1935.
- *B*. Dissociation of molecular addition compounds : H. C. Brown, 1944.
- *C*. Calculation of steric hindrance in *neo*pentyl derivatives : C. K. Ingold, 1946.
- *D*. Calculation of activation energy in diphenyl racemization : F. Westheimer, 1947.
- *E*. Quantitative treatment of rates : R. Taft, 1953.

It would be of little value to debate whether steric or polar effects are of greater importance to the organic chemist. Both factors are important and both factors must be utilized in developing a satisfactory understanding of the behaviour of organic substances.

The Chemical Society

**'SPECIAL PUBLICATION' NO. 2**

# PEPTIDE CHEMISTRY

The following Papers, presented at a Symposium held on March 30th, 1955, have been published, with a report of the discussion, as No. 2 in the Society's series of "Special Publications."

1. "Deuterium Exchange between Peptides and Water," by K. Linderstrøm-Lang (Carlsberg Laboratorium, Copenhagen).

2. "New Aspects of the Chemistry of Peptide Synthesis from *N*-Carboxy-α-amino-acid Anhydrides," by C. H. Bamford (Courtaulds Ltd., Maidenhead).

3. "The Synthesis of Cystine Peptides with Special Reference to Oxytocin," by V. du Vigneaud (Cornell University Medical College).

4. "The Use of Carboxypeptidase for End-group and Sequence Studies in Polypeptide Chains," by J. I. Harris (University of California and Carlsberg Laboratorium, Copenhagen).

5. "The Use of Mixed Anhydrides in Peptide Synthesis," by G. W. Kenner (University of Cambridge).

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*See April issue for the first list of additions*

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